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13 Seed

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In the world market, cottonseed production and processing ranks second (17.3% of world oilseed production) among the five major oilseeds, which include soybean seed, cottonseed, sunflower seed, peanut seed, and rape seed (Fig. 13-1; van Waalwijkn van Doorn, 1982; Anon., 1982a). The USA, USSR, People's Republic of China, India, and Pakistan produce approximately 75.3% of the 27.8 Mt of the world cottonseed supply (Table 13-1). Cottonseed is processed into four major products—oil, meal, hulls, and linters. These products and their many uses are summarized in Fig. 13-2 (Anon., 1950, 1978a). Cottonseed yields approximately 16% crude oil, 45% meal, 9% linters, and 26% hulls, with losses of 4% due to handling and processing (Kromer, 1977; Carter et al., 1979). World production of oil and meal (44% protein) for 1981/1982 were predicted to be 3.5 and 8.2 Mt, respectively (Mathews, 1981). In 1981/1982, the USA was expected to process 0.9 and 2.1 Mt of oil and meal, respectively.

Table 13-1. Cottonseed production by major producers.†

	1981/1982 Preliminary production	
	Mt	%
USSR	4 950	17.8
PRC	5 936	21.4
USA	5 803	20.9
India	2 750	9.9
Pakistan	1 470	5.3
Total	20 909	75.3

† From van Waalwijkan van Doorn, 1982; Anon., 1982a.

13-1 COMPOSITION

13-1.1 Major Constituents

Electron microscope photographs of cottonseed show them to contain a number of distinct structural features, including the following: (a) nuclei; (b) spherosomes, fat storage bodies; (c) protein bodies that contain the storage proteins, globulins; (d) globoids, phytin particles, in the protein bodies; (e) the cellular cytoplasm, in which structures (a) to (d) are embedded with nonstorage proteins, enzymes, and other particulates important to the physiology of the seed; (f) gossypol glands; and (g) cell walls (Fig. 13-3; Yatsu, 1965; Englemen, 1966; Dieckert and Dieckert, 1972, 1976). The fact that storage constituents are in compartments has enabled the development of techniques to separate them during the processing of cottonseed to feed and food ingredients.

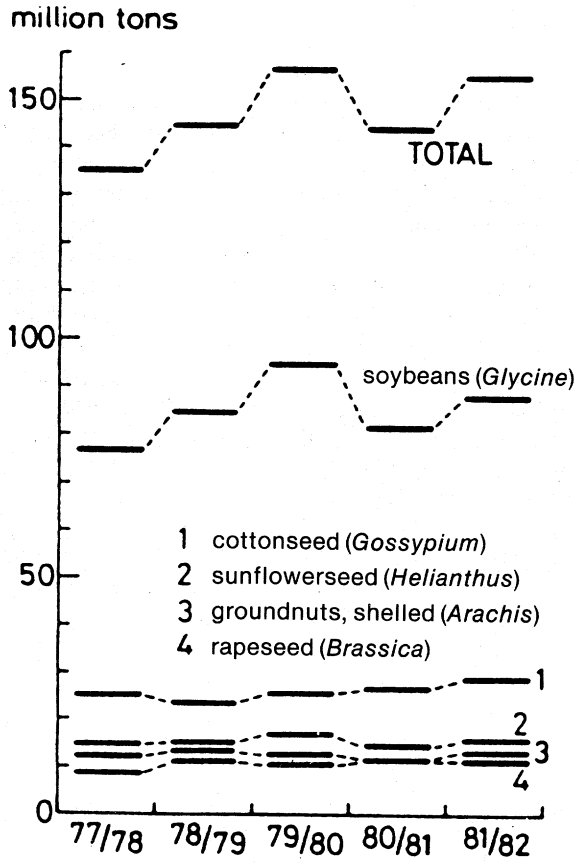


Fig. 13-1. World production of major oilseeds (van Waalwijkn van Doorn, 1982; Anon., 1982a).

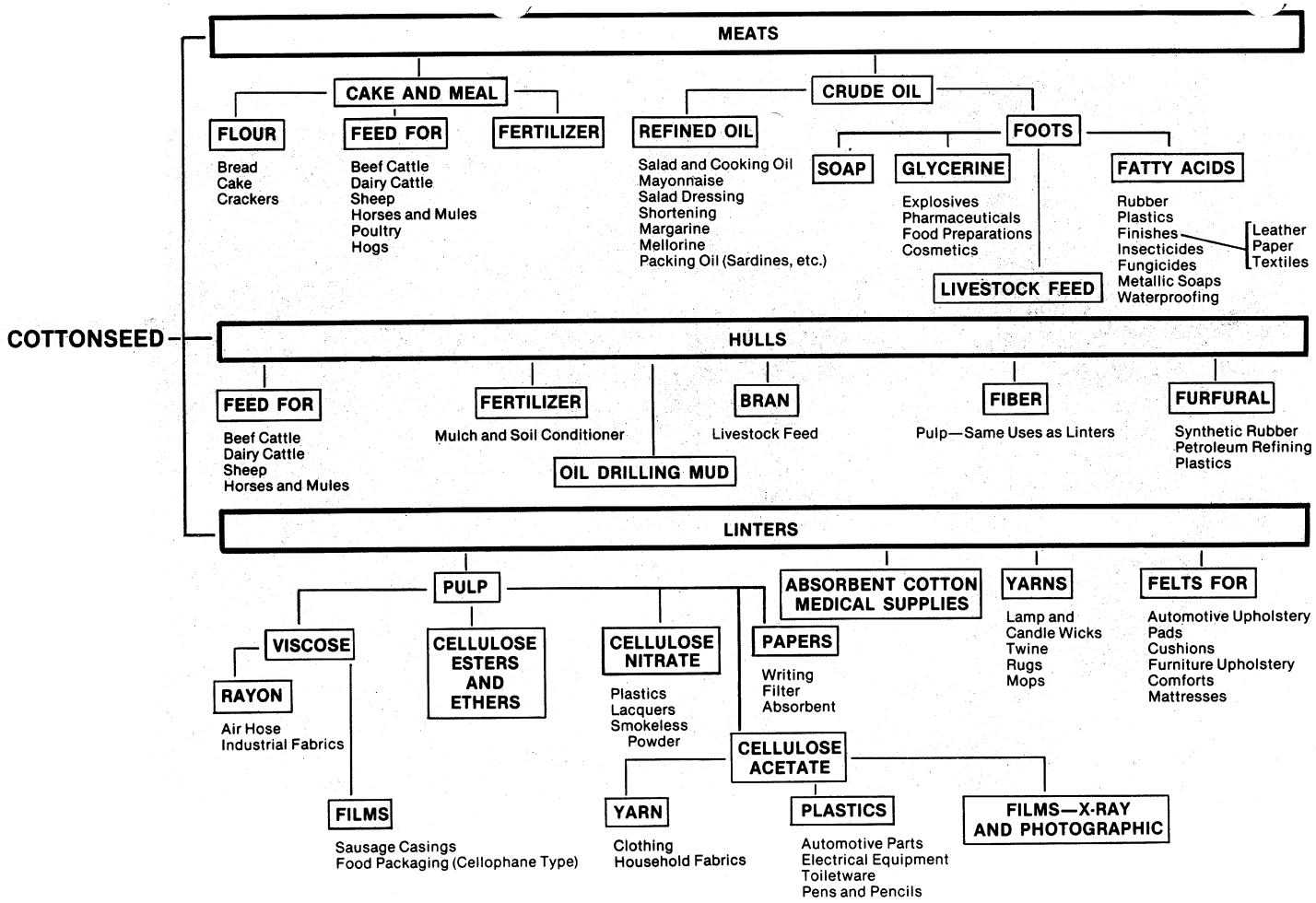


Fig. 13–2. Cottonseed products and their many uses (Anon., 1950, 1978a).

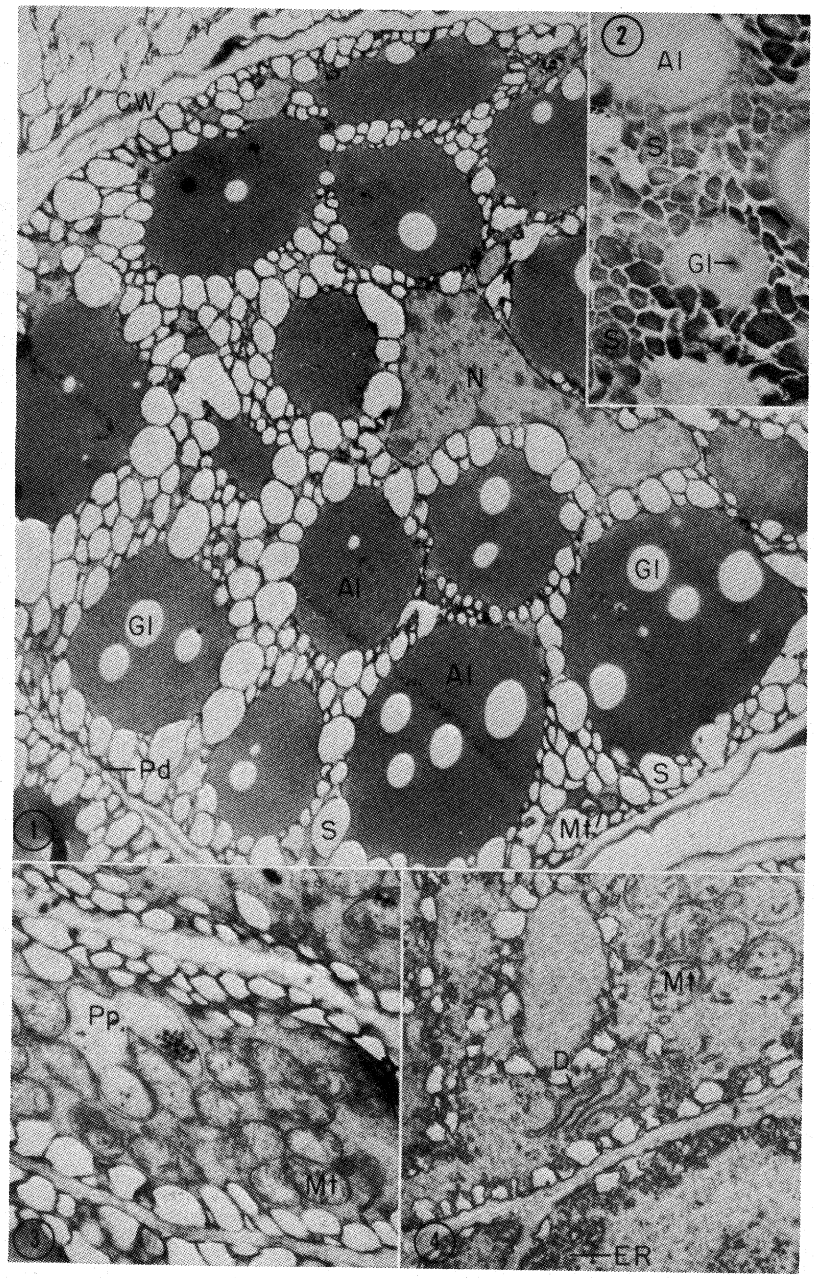


Fig. 13–3. Ultrastructure of cotyledonary tissue (Yatsu, 1965). 1) A typical spongy parenchymal cell from the cotyledon of a dry cottonseed fixed with lithium permanganate, × 7000, and 2) osmium tetroxide fixation, × 7000. 3) Provascular region of cotyledonary tissue fixed with lithium permanganate, × 12 000. 4) Dictyosome profile of (3) fixed with lithium permanganate, × 16 000. Al, aleurone grains; CW, cell wall; D, dictyosome; ER, endoplasmic reticulum; Gl, globoid; Mt, mitochondria; N, nucleus; Pd, plasmodesmata; Pp, proplastid; S, spherosome.

The breeding and production of cotton have traditionally been guided by considerations of fiber yield and quality. Until recently, seed characteristics, except for viability and vigor of planting seed, have generally been ignored. Competition from other seed sources in the oil and feed industries, and the developing prospect of using cottonseed as a food, have increased the awareness of the potential importance of cottonseed to food and feed reserves of the world. As a result, the National Cottonseed Products Association, Inc., Memphis, Tennessee, (Anon., 1980), an association of cottonseed processors, developed nine objectives relating to cottonseed quality improvements (Table 13–2). Research on cottonseed has made it clear that many factors affect their composition. Efforts to improve cottonseed composition must be accomplished without sacrifice to fiber quality and yield or planting performance.

Table 13–2. Identified goals relating to cottonseed quality.†

Goal	Needed change
Increase seed yield per acre	Concern for availability of cottonseed for crushing. Increasing acreage as well as total seed cotton yield per acre are the only two approaches acceptable to the entire cotton industry. The results would be increased yields of fiber, seed, oil, and protein per acre.
Increase oil percentage	Economically important to have seed with high percentages of oil. Variety tests show three percent or more variation between genetic lines which could be used to select for increased oil percentages in seeds of future cultivars.
Eliminate cyclopropenoid fatty acids	Biologically active fatty acids present in minute quantities of cottonseed oil. Although they are no health hazards, it is best to eliminate these fatty acids genetically.
Eliminate or reduce seed gossypol	As well as affecting animal performance when cottonseed meal is used as feed, gossypol pigments discolor oil so that additional processing, refining, and bleaching are necessary, and reduces the nutritional value of the meal when it binds to lysine. Cotton breeders have developed plants producing glandless (essentially gossypol-free) cottonseed.
Increase protein percentage	Increased seed protein percentage would improve the desirability of meal. Variety tests have shown that protein levels can be influenced by genetics.
Increase lysine content	Increased lysine in the protein would improve seed meal nutritional value. It should be possible for plant breeders to select lines capable of providing higher levels of lysine per unit of protein.
Improve mycotoxin resistance	Mycotoxins have cost both producers and crushers heavily. Cultural practices are available to reduce aflatoxin levels in seed cotton. Selection of resistant plants followed by a simple breeding program may be the start of controlling this in insidious production and crushing problems.
Insure production of non-shattering seeds	The ideal seed coat should protect the seed from mechanical damage and the subsequent deterioration of the kernel.
Reduce linter percentage	Introduction of varieties with higher seed linter level than are now common is not acceptable to the processes of the cottonseed crushing industry.

† From Anon. (1980).

Research shows that cultivar, location, year, and their interactions are highly significant sources of variation associated with cottonseed composition (Pope and Ware, 1945; Bailey, 1948; Stansbury et al., 1953a, b, 1954, 1956; Cherry et al., 1970; Pandey and Thejappa, 1975; Turner et al., 1976a, b; Lawhon et al., 1976; Cherry et al., 1978a, b, 1979c, 1981a, b; Cherry and Berardi, 1983; Cherry, 1983; Kohel and Cherry, 1983). Breeding and agronomic practices can affect both the physical and chemical properties of cottonseed, and thereby influence the planting seed quality and the efficiency of their processing into oil, feed, and food products without affecting fiber quality.

The composition of cottonseed and the effects of genotype and environment are summarized in Table 13–3 (Cherry et al., 1978a, b, 1981a, b; Cherry, 1983; Cherry and Berardi, 1983). Cottonseed are excellent sources of both oil and protein because of the high composition of these constituents and their fatty acid and amino acid qualities (Tables 13–4 and 13–5; Cherry et al., 1978a, b, 1981a, b; Cherry, 1983; Cherry and Berardi, 1983).

McMichael (1959, 1960) caused considerable excitement in the cottonseed industry when he reported that cottons with seed free of gossypol could be developed by selection for recessive alleles at two loci. Reviews on the genetic development of glandless cottons were presented by Hess (1976, 1977a). Progress has been made in developing glandless cottons that yield competitively with glanded cottons in fiber and seed (Phelps et al., 1979). The protein and oil compositions of seeds of glandless cultivars are at least comparable or exceed those of glanded cultivars (Lawhon et al., 1976; Cherry et al., 1978a, b, 1981a, b; Cherry, 1983; Cherry and Berardi, 1983).

Table 13–3. Summary of mean values of seed quality traits of cultivars grown at various Texas locations in 1974.†

Quality trait	Cultivars across locations	Locations across cultivars
Hulls†	37.2 –40.0	36.6 –44.8
Kernel‡	49.0 –50.9	43.5 –53.4q
Linters‡	9.9 –11.6	9.9 –12.4
Seed index§	9.3 –10.6	8.6 –11.1
Oil¶	23.6 –25.0	23.2 –25.7
Protein¶	25.6 –27.5	25.6 –27.6
Total sugars#	6.8 – 7.0	6.7 – 7.0
Ash#	4.4 – 4.5	4.1 – 4.9
Crude fiber#	2.1 – 2.2	2.0 – 2.3
Free gossypol#	0.74– 0.87	0.75– 0.86
Total gossypol#	0.90– 0.99	0.88– 1.00
N-solubility††	96.4 –97.9	96.5 –98.1
Cyclopropenoid fatty acids‡‡	0.97– 1.04	0.82– 1.18
ϵ-Free lysine§§	3.17– 3.21	3.12– 3.26

† From Cherry et al., 1978a, b.
‡ % of seed.
§ Number of ginned seed/100 g.
¶ %, moisture, lint-free seed basis.
# %, moisture-free kernel basis.
†† % of protein soluble in 0.02 *N* NaOH.
‡‡ %, oil.
§§ g/100 g fat-free flour.

Despite many years of interest in improving cottonseed quality, we find little continuity of either interest or research programs. Research on the quality of cottonseed, other than the breakthrough of gossypol-free seed, has not had an impact on the improvement of cotton cultivars; i.e., little has been done to utilize the wealth of information on compositional variability to improve cottonseed quality. For example, Pope and Ware (1945) found oil to range from 16.1 to 26.7%, and protein to range from 20.5 to 26.8% in cottonseed. Thirty years later, Lawhon et al. (1976) found the range of oil to be 16.5 to 25.6% and protein to be 19.6 to 24.0% in cottonseed. Turner et al. (1976a) reported average values of 19.6% for oil, and 24.8% for protein in cottonseed. Similar oil and protein values of 23.2 to 25.7%, and 25.6 to 27.6%, respectively, in cottonseed were reported by Cherry et al. (1978a, b, 1981a, b).

Evidence that cottonseed composition can be improved is shown by the breeding program of the Acala SJ series in California (Cherry et al., 1981a;

Table 13–4. Fatty acid composition of oil from glanded cottonseed kernels of cultivars grown at various Texas locations in 1974.†

Fatty acid	Range	Mean
	%	
Myristic (C14:0)	0.68– 1.16	0.82
Palmitic (C16:0)	21.63–26.18	23.68
Palmitoleic (C16:1)	0.56– 0.82	0.65
Stearic (C18:0)	2.27– 2.88	2.55
Oleic (C18:1)	15.17–19.94	17.41
Linoleic (C18:2)	49.07–57.64	54.54

† From Cherry et al. (1978a, b).

Table 13–5. Amino acid composition of fat-free flour of cottonseed from cultivars grown at various Texas locations.†

Amino acid	Range	Mean
	g/100 g sample	
Alanine	2.00– 2.26	2.12
Valine‡	1.66– 2.61	2.20
Glycine	2.08– 2.35	2.21
Isoleucine‡	1.20– 1.86	1.59
Leucine‡	2.88– 3.84	3.12
Proline	1.83– 2.13	1.96
Threonine‡	1.42– 1.98	1.74
Serine	2.14– 2.59	2.40
Methionine‡	0.59– 0.96	0.74
Phenylalanine‡	2.62– 3.02	2.82
Aspartic acid	4.58– 5.25	4.93
Glutamic acid	9.46–11.15	10.53
Tyrosine	1.29– 1.68	1.51
Lysine‡	2.28– 2.63	2.46
Histidine‡	1.12– 1.62	1.41
Arginine‡	4.39– 6.42	5.43
Half cystine	0.70– 1.08	0.88
Total	44.34–50.82	48.02

† From Cherry et al. (1978a, b).
‡ Essential amino acid.

Table 13–6. Mean values of cottonseed quality traits of Acala cultivars grown at four California locations, 1975–1977.†

Quality factors§	Cultivars‡	
	SJ-2	SJ-5
Hull	41.60 a‡	36.89 b
Kernel	45.37 a	51.63 b
Lint	19.03 a	21.81 b
Quantity index	97.29 a	109.59 b
Grade	96.73 a	109.29 b
Oil	19.03 a	21.81 b
Protein	22.25 a	23.44 b
Free fatty acids	1.20 a	0.86 b
Free gossypol	1.03 a	0.73 b
Total gossypol	1.09 a	0.80 b
Phosphorus	0.94 a	0.88 b
LCP Flour:		
Free gossypol	0.03 a	0.02 b
Total gossypol	0.06 a	0.04 b

† From Cherry et al. (1981b) and Cherry (1983).

‡ Means among cultivars having the same letter are not significantly different to the Newman-Keuls multiple range test.

§ Hull, kernel, and lint are presented as % of seed; oil and protein are % of linted seed; free fatty acid is % of oil; free and total gossypol, and phosphorus, are % of kernels; and LCP flour protein and free and total gossypol is % of flour. All of these values are presented on an "as is" moisture value which was 9.35 and 9.08 for the 'Acala SJ-2' and 'Acala SJ-5' cottonseed, respectively; values that were not significantly different.

Cherry, 1983). This program increased both oil and protein in Acala cottonseed, and simultaneously reduced gossypol (Table 13–6). Other statistically significant improvements in seed quality include the following: (a) reduced portions of the seed as hull and linters, (b) decreased amounts of cyclopropenoid fatty acids, and (c) higher levels of essential amino acids and select fatty acids (Tables 13–7 and 13–8). These improvements are consistent with those identified as important to the cottonseed crushing industry (Table 13–2).

13–1.2 Gossypol and Other Polyphenolic Pigments

The polyphenolic pigment gossypol, 1,1′,6,6′, 7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl (2,2′-binaphthalene)-8,8′-dicarboxaldehyde, is contained within discrete bodies in leaves, stems, and roots, and seeds of cotton plants (Boatner et al., 1947; Adams et al., 1960; Markman and Rzhekhin, 1965; Berardi and Goldblatt, 1980). In this form, the pigment is called free gossypol, because it is readily extracted by 70% aqueous acetone. At least 15 gossypol pigments or derivatives have been identified in extracts of cottonseed or their oils and meals (Berardi and Goldblatt, 1980); only eight have been isolated and characterized.

Gossypol has three tautomeric structures, (a) the hydroxyaldehyde, (b) the lactol, and (c) the cyclic carboxyl forms (Fig. 13–4). These structures are

Table 13–7. Mean values of amino acids of flour from cottonseed of Acala cultivars grown at four California locations, 1975–1977.†

Amino acid	Cultivars	
	SJ-2	SJ-5
	g/100 g flour	
Alanine	2.00 a†	2.03 a
Valine§	2.10 a	2.14 a
Half-cystine	0.79 a	0.82 a
Arginine§	6.47 a	6.62 a
Lysine§	2.24 a	2.26 a
Histidine§	1.50 a	1.59 b
Tyrosine	1.54 a	1.54 a
Aspartic acid	4.84 a	4.84 a
Glutamic acid	10.38 a	10.40 a
Phenylalanine§	2.68 a	2.78 a
Glycine	2.10 a	2.12 a
Isoleucine§	2.55 a	2.57 a
Leucine§	2.96 a	3.01 a
Proline	1.92 a	1.95 a
Threonine§	1.64 a	1.67 a
Serine	1.41 a	1.43 a
Methionine§	0.64 a	0.66 a

† From Cherry et al. (1981b).
‡ Means among cultivars having same letter are not significantly different according to the Newman-Keuls multiple range test.
§ Essential amino acid.

Table 13–8. Mean values of fatty acids of oil from cottonseed of Acala cultivars grown at four California locations, 1975–1977.†

Fatty acid	Cultivars	
	SJ-2	SJ-5
	%	
Palmitic (C16:0)	23.32 a‡	22.69 b
Palmitoleic (C16:1)	0.72 a	0.64 b
Stearic (C18:0)	2.17 a	2.29 b
Oleic (C18:1)	16.63 a	17.26 b
Cyclopropenes	0.90 a	0.84 b

† From Cherry et al. (1981b) and Cherry (1983).
‡ Means among cultivars having the same letter are not significantly different according to the Newman-Keuls multiple range test.

markedly reactive, exhibit strongly acidic properties, and act as phenolic and aldehyde compounds. When dissolved in dilute aqueous alkali, they behave as strong dibasic acids to form neutral salts. In alcoholic solution, gossypol is extremely sensitive to oxidation. The phenolic groups of gossypol react readily to form esters and ethers. The aldehyde groups react with amines to form Schiff bases, and with organic acids to form heat-labile compounds; gossypol reacts with the aromatic amines of aniline to form dianilinogossypol which can be analyzed quantitatively (Pons, 1977; Berardi and Goldblatt, 1980).

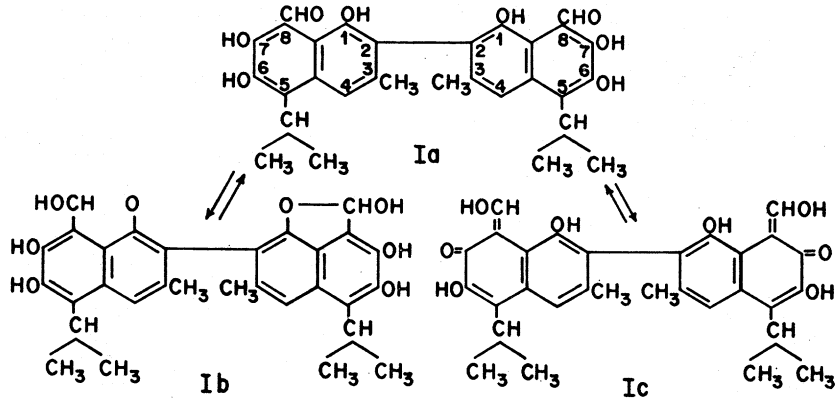
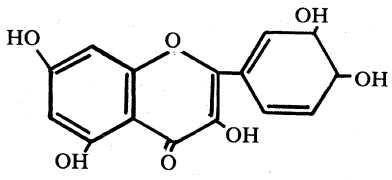


Fig. 13–4. Tautomeric structures of gossypol (Berardi and Goldblatt, 1980).

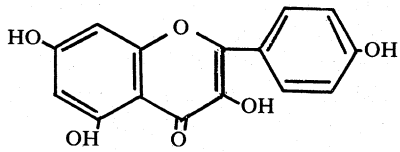
Since gossypol is highly reactive with other cottonseed storage constituents during processing, it is responsible, in part, for (a) reduction, through binding, of the biological availability of lysine, and thus the nutritive quality of the meal proteins; (b) adverse physiological effects in nonruminants; and (c) production of dark-colored pigments in the oil and meal that are not removed in conventional refining and bleaching operations (Berardi and Frampton, 1957; Pons et al., 1959; Smith, 1972; Jones, 1981; Berardi and Goldblatt, 1980; Bressani et al., 1980; Cherry, 1983).

Adverse physiological effects may be counteracted by making free gossypol a bound form during processing, by the use of heat and certain minerals, especially iron salts (Anon., 1966; Berardi and Goldblatt, 1980). To quantitatively extract bound gossypol from cottonseed, it first must be hydrolyzed to the free form with compounds such as oxalic acid (Pons, 1977). Total gossypol is then determined as the sum of the free and bound gossypol and gossypol-like pigments extracted after oxalic acid hydrolysis.

Thin layer chromatography and gel filtration have been used to fractionate seven major flavonoid pigments in solvent extracts of cottonseed products (Blouin and Cherry, 1980; Blouin et al., 1981a, b, 1982). These major flavonoids have been tentatively identified and are listed in Table 13–9. Diagnostic ultraviolet-visible spectral analysis indicated that flavonoids 1, 2, 5A, 5B, and 6 were 3-o-glycosides of quercetin, while flavonoids 3 and 4 were 3-o-glycosides of kaempferol.



quercetin



kaempferol

Table 13–9. Cottonseed flavonoids.†

Designation	Tentative identification	Characterization method‡
1	quercetin 3-0-neohesperidoside (2-0-α-L-rhamnosyl-β-D-glucoside)	1,2,4
2	quercetin 3-0-glucoglucoside	1,2
5B	quercetin 3-0-robinoside (6-0-α-L-rhamnosyl-β-D-galactoside)	1,2
5A	quercetin 3-0-rutinoside or rutin (6-0-α-L-rhamnosyl-β-D-glucoside)	1,2,3
6	quercetin 3-0-glucoside or isoquercetrin (β-D-glucoside)	1,2,3
3	kaempferol 3-0-neohesperidoside (2-0-α-L-rhamnosyl-β-D-glucoside)	1,2,4
4	kaempferol 3-0-glucoside	1,2

† From Blouin et al. (1981).
‡ (1)—Ultraviolet—visible diagnostic spectral analysis.
(2)—Hydrolysis and TLC of aglycones and sugars.
(3)—Chromatographic mobility with standards.
(4)—NMR spectra.

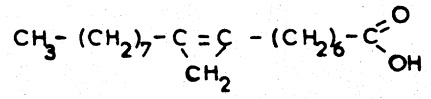
When cottonseed products are used in foods, gossypol and flavonoids contribute to a serious discoloration problem. If plant protein products are to be used in food, the color problems must be solved. Yellow colorations are caused by flavonoids, and brown discoloration is mainly bound gossypol. A knowledge of the nature of the bonding between the components causing color and proteins should lead to the development of better methods for prevention or elimination of these problems.

13–1.3 Cyclopropene Fatty Acids

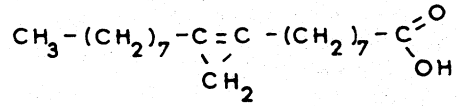
Cottonseed oil contains sterculic and malvalic acids; 18 and 17 carbon cyclopropenoid fatty acids (CPFA), respectively, which contain one double bond at the site of a propene ring, either at the 9, 10, or 8, 9 position (Fig. 13–5). The cyclopropene ring is chemically and physiologically reactive (Phelps et al., 1965; Allan et al., 1967).

The cyclopropene ring reacts with sulfur dissolved in carbon disulfide to produce a chromogen (Halphen reaction) that allows colorimetric quantitation of the CPFA (Deutshoman and Klaus, 1960; Sheehan et al., 1972). Acid titration, in which halogen acids (primarily HBr) are added to the double bond of the cyclopropene ring, is another method for the quantitation of these fatty acids (Mayne et al., 1966; Feuge et al., 1969). Derivatives of CPFA have been quantitated by gas-liquid and high performance liquid chromatography (Fisher and Schuller, 1981; Bianchini et al., 1982; Fisher and Cherry, 1983).

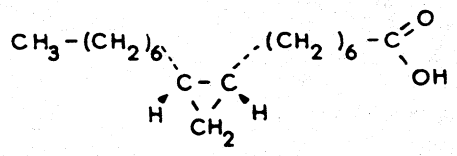
Poultry and animal feeding studies have shown CPFA to change the ratio between stearic and oleic acids; as a result, CPFA cause the hardening of fats in egg yolk and milk (Johnson et al., 1967; Roehm et al., 1970; Bickerstaffe and Johnson, 1972). The fatty acids bind to the thiol groups of



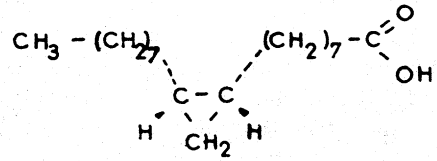
C18:CE malvalic acid (8,9-methylene-8-heptadecenoic acid)



C19:CE sterculic acid (9,10-methylene-9-octadecenoic acid)



C17:CA cis-8,9-methylenehexadecanoic acid



C19:CA dihydrosterculic acid (cis-9,10-methyleneoctadecanoic acid)

Fig. 13–5. Cyclopropenoic and cyclopropanoic fatty acids (Bianchini et al., 1982).

acyl desaturase, a complex of enzymes involved in the unsaturation of stearic acid to form oleic acid (Kircher, 1964; Allan et al., 1967). The CPFA also alter permeability of the vitelline membrane of the egg yolk, which leads to diffusional changes shown by altered concentrations of water, fat, and proteins in the yolk. They also affect concentrations of iron and non-protein nitrogen compounds in the white, causing them to become discolored (Phelps et al., 1965). Jones (1981) reviewed other possible effects of CPFA ingested by poultry and animals, that have been reported by a number of investigators. There is evidence that the CPFA act as cocarcinogens with aflatoxins in trout, although feeding studies with rats to determine the cocarcinogenicity of CPFA and aflatoxins have not been conclusive.

In the process of oil production from cottonseed, the deodorization and hydrogenation steps greatly reduce the Halphen response normally detected in crude oil (Phelps et al., 1965). Calculations of human consumption, based on domestic disappearances of cottonseed oils, proportions of vegetable fat in the U.S. diet, and statistics on food consumption, indicate human CPFA intakes of at least one order of magnitude lower than that used in almost all studies with laboratory animals reported in the available literature (Jones, 1981). This finding is consistent with the ob-

servation that no deleterious reactions have been noted in the more than a century that cottonseed oil has been available commercially as a vegetable oil for human use, although the reactivity of CPFA is such that it should be given priority in cottonseed improvement efforts.

13-2 SEED DEVELOPMENT

13-2.1 Cultural Conditions

Reproductive development starts in the early stages of plant growth, although vegetative growth predominates during the early stages of development. Gradually, the partitioning of growth is shifted increasingly toward reproductive and away from vegetative development. Finally, vegetative growth is essentially arrested, at least until most bolls are advanced in development. Then, if environmental conditions are favorable, a second cycle of vegetative and reproductive growth is initiated. This regrowth phenomenon is a liability to successful crop production management in most areas of the cotton production zone.

Because of the indeterminant flowering pattern of cotton, fruit forms are initiated over a period of several weeks. Basically, compositional changes during cottonseed development and ripening are like those of other seeds (Altschul, 1948; Grindley, 1950; El-Nockrashy et al., 1976; Elmore and Leffler, 1976; Kajimoto et al., 1979). Even if meteorological conditions are generally considered to be "ideal" for crop growth, many aspects of the environment interact to produce a continuously varying system in which seed development takes place. Seeds in relatively early bolls are nourished through the high photosynthetic capability of a comparatively young plant, while those in later bolls are nourished by the lesser capacity of the more aged canopy. These contrasts between seeds of early and late bolls are made even more striking by shifting weather patterns as summer advances to early fall (Meredith and Bridge, 1973; Benedict et al., 1976; Leffler et al., 1977, 1978). Seasonal changes in seed size and composition are consistent both with the effects of cool night temperatures (Gipson and Joham, 1969; Gipson and Ray, 1970) of late-season development and with the declining photosynthetic activity of an aging leaf structure (Leffler, 1980). For example, Gipson and Ray (1970) showed that percentages of oil tended to respond hyperbolically to temperature, with the optimum being near 20°C. Stansbury et al. (1954) examined eight cultivars at 13 locations for 3 years and found the correlation of percentage of oil to maximum temperature to be $r = -0.57$ (significant at the 0.01 level). Low temperature changed both the ratio among simple sugars and the time of their maximum accumulation in seed (Conner et al., 1972). The rates of accumulation of minerals and reserves were also altered by low temperatures (Kreig et al., 1973).

Evaluations of the planting quality of cottonseed obtained from stratified harvests (repeated, frequent harvests of seed cotton) at Stoneville, Mississippi, have consistently shown a nearly linear decline with later

periods of development and, consequently, times of harvest. As indicated by Leffler et al. (1978), the initial and final stand counts were reduced with seeds formed late, and seedling growth rates and yielding capability of the seedlots were similarly reduced. The very early study of Simpson and Stone (1935) suggested that seed formed late may not germinate as well as those formed early. The contributions of the environment to the development of the quality of cottonseed can be highly significant (Peacock and Hawkins, 1970). Efforts to manipulate the reproductive development of cotton must therefore consider the resultant effects on seed quality.

Within a production environment, the processes of seed development can be adversely affected by meteorological extremes. Leffler (1976) described the temporary cessation of dry matter and protein accumulation by cottonseed during a period of high rainfall and low solar radiation.

Maturing cottonseed responded to elevated nitrogen fertilization by synthesizing additional storage proteins (Sood et al., 1976; Leffler et al., 1977). The degree to which these increases in storage proteins occurred depended on the time during the maturation period that fertilizer was added. If environmental conditions are adequate to influence the patterns of seed composition, it is reasonable to conclude that they might also affect the normal synchrony of development during germination.

Stewart (1980) calculated from data of Leffler et al. (1977) that high nitrogen reversed the decline in oil, but not protein. In further studies, major environmental differences during two seasons at College Station, Texas, influenced intraseasonal variation (Kohel and Cherry, 1983). Weather conditions affected seed development, differentiation, and energy demands within cotton plants as the growing seasons progressed. Seed decreased in weight and in their relative concentrations of oil and free gossypol, but increased in protein with progressively later harvests. Significantly, there appears to be a marked contrast between Texas and Mississippi in the seasonal responses of oil and protein. In numerous evaluations of seed quality, seed of some cultivars consistently rate at or near the top, while seed of other cultivars consistently rate poorly. Since the quality of seed is not usually a characteristic for which direct genetic selections are made, these observed differences among cultivars are probably the natural results of unselected characteristics. There are indications that improvement in seed quality might be made through breeding efforts. El-Zik and Bird (1969) reported that the ability to produce a stand of cotton was a quantitatively inherited trait that could be enhanced through breeding. Similarly, Hess (1977b) described the genetic enhancement of seed density, which is associated with cottonseed quality.

The production of a seed crop is dependent on the leaf canopy. Consequently, anything that influences either the rate or the duration of the canopy development may also affect the quality of the seed that the plants produce. One management factor is the establishment of a population density for the crop. Both vegetative and reproductive developments of the cotton crop are highly influenced by the density of the population of plants. By arranging a fixed population of plants into different competitive pat-

terns, Walhood and Johnson (1976) produced significantly different rates and durations of crop development. The varying competition among plants also influenced the times at which the plants initiated reproductive development. Thus, because of these effects of flowering patterns, earliness, and cutout, the crops from the various spatial patterns matured under different environmental conditions. Both Caldwell (1962) and Maleki (1966) described reductions in the quality of cottonseed produced at above-normal plant population densities. These declines in quality may result from shifts in canopy development related to the density of populations.

13-2.2 Chemical Treatments

Throughout the normal cotton production season, the crop canopy is frequently subjected to numerous chemical treatments. The majority of these applications are made in the effort to control insects, although late-season applications with growth terminators or defoliants are also common. Additionally, there has recently been considerable interest in the early- or mid-season application of plant growth regulators (e.g., 1,1-dimethyl piperidinum chloride, or mepiquat chloride) to manipulate the development of the canopy (Anon., 1978b, 1979; Morgan, 1979).

While it is often acknowledged that canopy development is influenced by the response of plants to insects, it is often overlooked that cotton plants may also respond to the application of chemical insecticides. For example, Brown et al. (1962) reported that accumulation of yield was delayed in cotton treated with methyl parathion. Roark et al. (1963) later attributed this delay to a direct effect of the chemical on the metabolism of the cotton plants. Although the effects of insecticide applications on seed quality have not been evaluated, such an effect could be identified, if for no other reason than the shift in crop maturity.

Growers frequently include harvest-aid and defoliant chemicals among their cotton management practices; these practices have recently been reviewed by Cathey (1979). When properly applied, these chemical treatments can benefit the producer. If, however, applications are made prematurely—when too many bolls have yet to open—these chemicals can lower the quality of cottonseed, particularly those in the second harvest (McMeans et al., 1966; Cathey, 1979).

13-3 POSTMATURATION AND PREHARVEST

13-3.1 Field Environment

As developing bolls complete their filling periods, they progress to the maturation phase. Completion of the maturation phase is identified initially by the dehiscence of the boll and finally by the drying and fluffing-out of the seed cotton. Since seed cotton is usually harvested only once or twice, many open bolls remain in the field for a considerable time before harvest.

During this postmaturation, preharvest interval, the seed cotton is exposed to weathering. This exposure can hurt the quality of the seed for planting, especially in the humid areas of the U.S. Cotton Belt. Weathering deterioration is of little consequence in the arid cotton production zones, because of the absence of the moisture required to support biological activity in the seed.

The first bolls to open are usually low and inside the canopy; they are exposed to prolonged weathering and dry slowly because of the atmospheric humidity "trapped" by the foliage. Further, in those areas where cotton is harvested only once, the duration of the exposure is greater. Simpson and Stone (1935) provided one of the earliest documentations of the association between moisture and the deterioration of seed quality. Cottonseed exposed to weathering deterioration usually contain elevated amounts of free fatty acids, exhibit reduced levels of germinability and seedling vigor, and often have an off-colored (greenish or greenish-brown, rather than creamy white) embryo (Altschul, 1948). Weathered seedlots also often produce a high number of abnormal seedlings, particularly those in which cell division in the root tip does not occur normally (Wiles and Presley, 1960; Hunter and Presley, 1963).

A variety of yellowish, greenish or greenish-brown compounds, including glandular terpenoids (gossypol), flavonoids, and flavonol glucosides are present in cottonseed, which may contribute to autodestruction and discoloring processes during their weathering (Halloin and Bell, 1979; Halloin, 1981, 1982). Nonglandular terpenoid aldehydes have been synthesized in glandless cottonseed containing high moisture.

Continued exposure of seed cotton to high levels of moisture produces conditions that support the resumption of high metabolic activity in the opened bolls, especially early in the fall when temperatures are relatively high. Under these conditions, those seed that do not possess hard seedcoats become hydrated and initiate some phases of normal germination physiology, particularly lipolysis. This metabolism is not usually sustained, both because seeds do not remain hydrated and because residual biochemical dormancy may suppress further development. When open bolls remain unharvested for an extended time, through repeated cycles of wetting and drying, however, the deleterious effects of this exposure to the elements accumulate (Cherry et al., 1978a, b).

13–3.2 Microorganism Infections

In addition to the germinative or pre-germinative deterioration described above, the seed and boll infections by pathogens also contributes to losses of quality of seed in the field. Development of internal infection by microorganisms is also associated with the exposure of cottonseed to moisture and weathering conditions in the field. In the relatively moist eastern areas of the U.S. Cotton Belt, several species of fungi and bacteria have been isolated from field-weathered seed. Fungal infections by *Aspergillus niger*

(Link ex Fries) and species of *Alternaria, Colletotrichum, Diplodia, Fusarium* and *Rhizopus* were indicated by Halloin (1981, 1982) to be common; bacterial infections in this zone include those caused by *Bacillus, Pseudomonus,* and *Xanthomonas.* Conversely, in the more arid western areas of the U.S. Cotton Belt, only the incidence of the aflatoxin-producing fungus *A. flavus* (Link ex Fries) received prominence in the review by Halloin (1981). Not even in arid zones can the influence of moisture be separated from the development of this fungus, however, since Russell et al. (1976) described the effects of water management practices on its incidence.

The symptoms of seed deterioration due to the direct effects of weathering—increases in seed free fatty acids, coupled with decreases in germinability and the suppression of seedling vigor—are also observed in the indirect effects brought about by pathogenic development. Roncadori et al. (1971, 1972) reported significantly positive correlations between the free fatty acid percentage and the fungi in the embryo. Each of these seed quality determinants increases with length of field exposure prior to harvest (Roncadori et al., 1972). Consequently, Halloin (1981) suggested that both lipolysis and other aspects of seed quality deterioration resulted from the combined actions of seed (direct) and microbial (indirect) systems. Whatever may be the main reasons for it, the fact remains that, especially in humid cotton production zones, quality deterioration is proportional to the period of time that elapses between boll opening and seed harvest.

The harvest of cottonseed for planting and processing purposes should obviously be timely to minimize any field deterioration of seed quality. Stratified harvest studies in the Mississippi Delta indicated a progressive reduction in quality of planting seed; even in the absence of weathering deterioration (Leffler et al., 1978). Consequently, seed for use in planting should probably be obtained from only the first two-thirds of the crop to mature. Similarly, because of the rapidity of field deterioration once the seed have been exposed to moisture, any seed that have been exposed to fall rains should (ideally) be removed from the processing chain that provides planting seed, feed, and food products.

13-3.3 Aflatoxins

Throughout the world, aflatoxins are the only contaminants of feeds and foods routinely monitored. The level of aflatoxin contamination cannot exceed 10 μg/kg (Pons and Franz, 1978; Lee and Goldblatt, 1981; Ciegler et al., 1981). Aflatoxins B_1, B_2, G_1, and G_2 are most commonly found in food and feed commodities contaminated by *A. flavus*; M_1 and M_2, metabolic byproducts produced by cows ingesting feeds containing B_1, are found in milk and dairy products. The structures and physicochemical properties of aflatoxins are presented in Fig. 43-6. Spectrophotometric properties enable the precise detection of aflatoxins after their separation by thin layer chromatography (TLC) and high pressure liquid chromatography (Lee and Goldblatt, 1981). In addition to

the chemical tests, products suspected of being contaminated by aflatoxin are further checked by a number of biological systems—including chick embryos, ducklings, rats, hamsters, guinea pigs, dogs, trout, catfish cells, brown bullhead, and others (Ciegler et al., 1981).

Swine, cattle, and poultry given feed contaminated with aflatoxin suffer hepatic necroses, fatty infiltration, bile duct proliferation, and

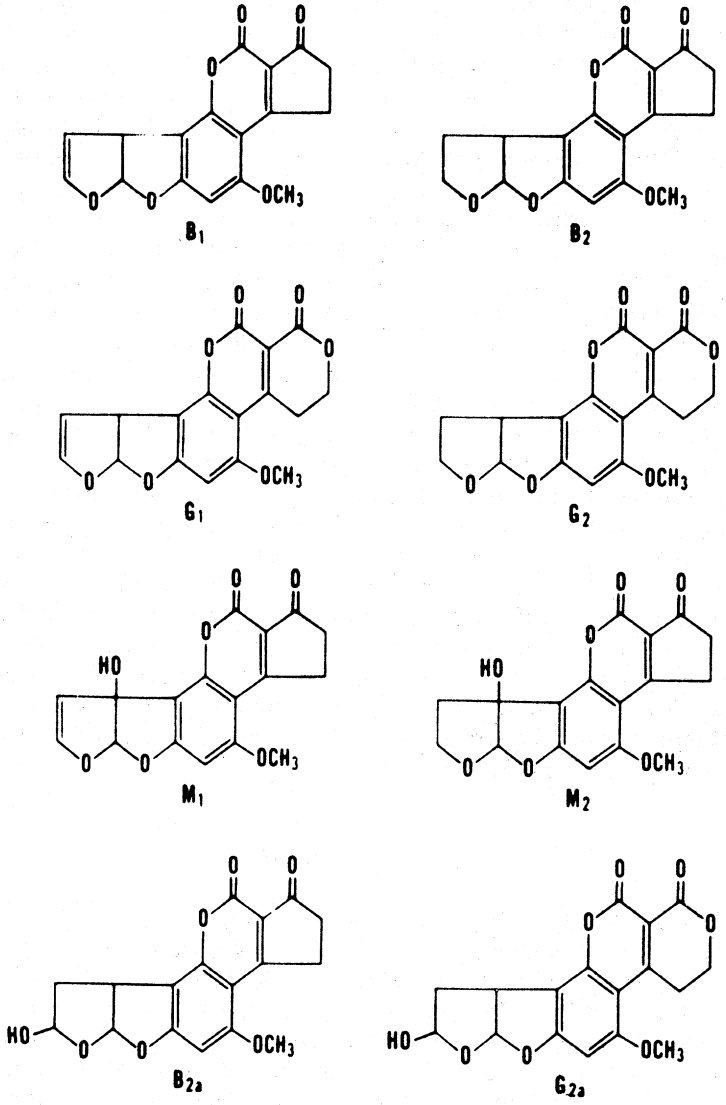


Fig. 13–6. Structures of aflatoxins (Ciegler et al., 1981).

hepatic failure (Newberne and Rogers, 1981). These acute toxicities of aflatoxins have been experimentally reproduced with laboratory animals. The principal target organ is the liver, although necrosis of spleen, pancreas, heart, and kidney occurs, and fatty infiltration of the latter two organs have been observed. Because of their acute toxicity and proven carcinogenic nature, aflatoxins have become a mycotoxin of utmost concern.

When considering factors affecting cottonseed composition, *Aspergillus* spp. (in particular, *A. flavus*) contamination and its production of alfatoxins ranks as the greatest cause of deterioration (Goldblatt, 1969). Because *Aspergillus* species were once considered storage molds, it was believed that aflatoxin contamination of seed arose primarily, if not exclusively, after harvesting. Factors that influence mold growth and toxin production on a commodity such as cottonseed are similar to other factors that affect seed quality: moisture, relative humidity, temperature, seed or substrate composition, competing microorganisms, and fungal strain. Moisture and relative humidity are probably the most important factors involved in fungal growth. It is now known that although *Aspergillus* molds can be a storage problem, they are also a field problem. In cottonseed, the problem is largely confined to a few locations in the far southwestern USA, and there it is primarily a field problem—exactly the reverse of what was thought a few years ago. The presence of aflatoxins in cottonseed and their products prevent the use of the contaminated seed in both feeds and foods.

As a result of these observations, Ramey (1974) presented a system that helped define factors affecting aflatoxin production during the maturation of bolls and seed of cotton. The system identified major research areas that could lead to an understanding of *A. flavus* growth and aflatoxin synthesis in cottonseed as follows: (a) determination of the origin and quantity of *A. flavus* inoculum in the field, (b) study of the environmental conditions leading to *A. flavus* infection of cotton bolls, (c) analysis of the regulatory mechanisms governing the production of secondary metabolites, and (d) development of additional information on genetic variability in *A. flavus* strains.

Contamination by aflatoxin in freshly harvested bolls of cotton can be detected by long wavelength ultraviolet light (Ashworth and McMeans, 1966); contaminated cotton has a bright greenish-yellow fluorescence (BGYF). This method has received much attention, and is being used to detect aflatoxin in the fiber of seed cotton. The highest concentrations of aflatoxin in seed were associated with unfluffed seed cotton. These findings stimulated studies to establish the mode of infection of cottonseed by toxin-causing fungi in the field. Only certain seed have been found to be aflatoxin-contaminated, even though select locks or entire bolls were *A. flavus*-infected. This selectivity might be explained if mold infection occurred during pollination when mold spores carried by insects may have intermingled with pollen (Lee and Russell, 1981).

13-4 HARVEST AND POSTHARVEST

13-4.1 Field Handling

The biological quality of cottonseed is maximum when they mature on the plant; handling of the seed during harvesting and processing subjects them to reductions in quality (Delouche, 1981). These quality reductions are caused by two types of factors: biological deterioration during storage and mechanical damage during processing and handling. Mechanical damage can occur at each operation, beginning with harvesting and continuing through the final processing step.

Seed can be damaged in harvesting if the equipment is not maintained, adjusted, and operated properly. Chief problems in the harvesting procedure are associated with excessive speed of operation and impact damage to the seeds (Colwick et al., 1972). Harvesting damage to cottonseed is usually characterized by cracked or broken seedcoats, often leaving portions of the embryo unprotected and exposed (Delouche, 1981).

13-4.2 Storage

Once the seed cotton has been harvested, it may be stored for a while before it is ginned. The conditions under which this storage occurs are major determinants of the desirability of this storage. In many ways the type of deterioration that may occur during storage of seed cotton is similar to that occurring in periods of adverse weather in the field prior to harvest. Consequently, high temperatures and moisture must be minimized during storage of seed cotton. Moisture is particularly important because if moisture in the seed mass is adequate to support elevated respiratory activity, the heat liberated can raise temperatures to extreme levels (Thomson, 1979). In his recent review of seed cotton storage, Baskin (1981a) concluded that field storage of seed cotton with greater than 10% moisture should not exceed 2 or 3 days to ensure the preservation of seed quality.

The module builder, which allows field storage of seed cotton prior to ginning, may transform the cotton industry by breaking the temporal connection between harvesting and ginning, allowing each operation to proceed at its own pace (Roberts et al., 1973; Paxton and Roberts, 1973; Curley et al., 1973; Kepner and Curley, 1976; Eickhoff and Willcutt, 1978; Wilkes, 1978; Cherry et al., 1979b, 1981a). Germinability, mycroflora, and free fatty acid content of high quality cottonseed are not affected significantly during module storage of seed cotton so long as seed cotton moisture remains below 12%. The oil and protein quality deteriorate regardless of seed moisture level, however. The moisture in trash such as leaves, soil, and branches causes localized "hot spots" that damage the seed. Temperatures that exceed 49°C during module storage indicate that seed are deteriorating and that the seed cotton should be ginned immediately. The addition of propionic acid to moist seed cotton stored in modules lessens these changes.

Good management during harvesting and close monitoring of the conditions of the seed cotton during storage are requisite to the use of modules.

13-4.3 Ginning

After the harvesting operation, the seed cotton is conveyed to the gin. Operations at the gin contribute to the mechanical damage observed in seedlots. Watson and Helmer (1964) found that approximately 1% seed damage was attributable to the cleaning, drying, and conveying operations, while an additional 5% damage was associated directly with the ginning operation. They also found that both seed moisture and ginning rate were directly proportional to the incidence of seed damage. The mechanical damage inflicted by gin saws is characterized by cuts or gashes in the seedcoat (Delouche, 1981).

Cottonseed from the gin are held in storage, where efforts must be made to minimize heat accumulation. Aeration of cottonseed stored in bulk should begin as soon as possible after ginning to reduce the heat remaining from the drying operations at the gin. The effects of bulk storage are similar to those described previously for module storage of seed cotton; the combined effects of elevated temperature and moisture can destroy a seedlot within a short period of time (Delouche, 1981; Baskin, 1981b).

13-5 PROCESSING

13-5.1 Planting Seed

The value of cottonseed used for planting is determined initially by their ability to germinate rapidly, support vigorous seedling growth, and produce an acceptable yield. Only insofar as it influences one or another of these principal determinants of seed quality does the seed's chemical composition merit consideration as a factor in planting seed quality.

Because gin-run cottonseed, taken directly from the gin, are still covered by fuzz fibers (linters), they tend to clump together into a mass. This aggregation of the seed effectively prevents controlled handling of single seeds during the planting process. To enhance their handling characteristics, cottonseed are subjected to one of several operations to remove the fuzz. Even though this delinting introduces an additional step to the processing of cotton planting seed, it is essential to the delivery of a product that is suitable for controlled handling. Several methods exist for delinting cottonseed, each falling into one of the three main categories of (a) mechanical delinting, (b) flame delinting, or (c) acid delinting (Delouche, 1981).

Mechanical delinting is a repetition of the ginning operation. This reginning is done with more and finer saws, spaced closely together, which act upon the seeds to remove most of the fuzz. Although mechanically delinted seed are less likely to aggregate than are gin-run seed, they do not separate

completely into single seeds because they are not completely free of fuzz. Additionally, mechanical delinting subjects the seed to another cycle of vulnerability to mechanical damage from the saws.

Flame delinting is a second-stage delinting operation, in that seeds which have first been mechanically delinted are then passed through an intense flame to singe most of the remaining fuzz. If conditions are adequately controlled, little deterioration in seed quality can be attributed to the flaming process. However, the tolerances to excessive heat are narrow, and conditions must be carefully controlled. The handling characteristics of flame-delinted seed are superior to those of mechanically-delinted seed, but are not usually as good as those of acid-delinted seed.

The third category of delinting procedures, acid delinting, produces the most completely delinted seed. There are three types of acid delinting, including (a) wet acid, (b) dilute wet acid, and (c) gas acid. In wet acid delinting, concentrated sulfuric acid is used to hydrolyze the fuzz. The dilute acid procedure introduces a dilute solution of sulfuric acid to the seed; this concentration is then effectively increased as the acid-wet seed are dried. Partial delinting is accomplished by lowering the initial concentration of sulfuric acid. The gas acid method employs gaseous anhydrous hydrochloric acid to degrade the fuzz. The degraded fuzz in all three processes are then removed by frictional abrasion in a buffer drum. Because delinting is complete, or nearly so, the handling characteristics of acid-delinted seeds are excellent, so precision metering during planting is possible.

Since acid can adversely affect seed quality, no matter which method of acid delinting is used, residual acidity must be removed, either by washing or by neutralization, or both. Seed quality problems arise when high temperatures occur, reaction times are too long, or the neutralization process is incomplete. Since acid can enter small cracks and holes in the seedcoats, complete removal of acid is particularly important, but difficult, in seedlots that have a high level of mechanical damage.

Once the cottonseed have been delinted, they are ready for the conditioning treatments that generally precede their use as planting seed. This conditioning process actually refers to the upgrading of a seedlot's performance through the removal of contaminants and some of the poorly performing seed from that seedlot. Two basic types of conditioning treatments are used in the processing of cotton planting seed. The first of these treatments is sizing by screening seed from debris on the basis of their length and width. Passage of seed through length/width separators enables the removal of contaminants that are outside the normal size range for cottonseed. Removal of immature or low-density seeds from the seedlot is also accomplished with the gravity table. This procedure, conducted on acid delinted seed, can significantly improve the germination performance of a seedlot by removing the smaller, weaker, lower density seeds.

Before cottonseed are used for planting purposes they often are coated with one or more seed treatments; these usually include a fungicide, and sometimes an insecticide as well. While these chemicals may enhance the field performance of a seedlot, especially under adverse conditions, they

can reduce the longevity of the seed. The chemicals have a degree of phytotoxicity which may be magnified in seedlots containing high levels of mechanical damage; consequently, storage of treated seed should be minimized except where seed are of highest quality (Thomson, 1979; Davis et al., 1981).

13-5.2 Oil Extraction

Extraction of oil from cottonseed involves the following steps: (a) elimination of leaves, twigs, pieces of bolls, and sand; (b) removal of fuzz fibers, (c) dehulling; (d) screw press extraction, solvent extraction or pre-press-solvent extraction; (e) solvent removal; (f) toasting; and (g) grinding or making into pellets (Harrison, 1977). The preparation and separation processes necessary to achieve maximum extraction of oils from various seed including cottonseed, are summarized in Fig. 13-7 (Cherry and Berardi, 1983; Cherry, 1983). The crude oil is warmed and treated with sodium hydroxide to enable removal of the soapstock. This refining process also removes the darker coloring materials, such as gossypol, leaving a clear yellow oil. Bleaching clay is used to remove any remaining colored substances. A winterization step at 3° to 4°C removes stearine, a material that turns the oil cloudy at 4° to 10°C. The compositions of feed-grade meals prepared by screw press, pre-press solvent, and direct solvent extraction methods are presented in Table 13-10 (Anon., 1982b); percentages of fatty acids in the oils extracted by these three processes were similar to those presented in Tables 13-4 and 13-8. Reviews of research programs having the objective of improving the competitiveness of cottonseed as a viable source

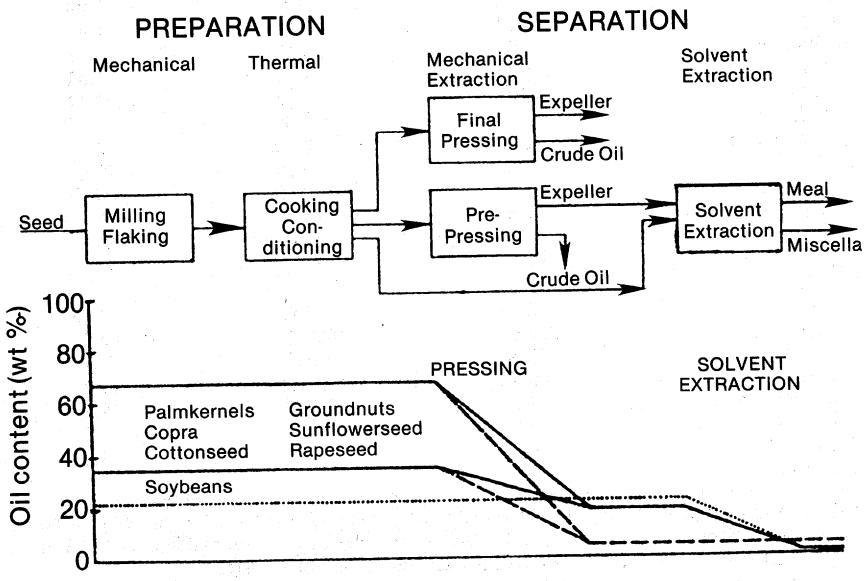


Fig. 13-7. Oil recovery processes (Cherry, 1983).

of edible oil products were presented by Cherry et al. (1981a, b), Cherry and Berardi (1983), and Cherry (1983).

Glandless cottonseed can be processed much more efficiently than glanded seed to high quality kernels, oil, and meal products (Fig. 13–8;

Table 13–10. Adjusted mean analytical composition of cottonseed meal by oil extraction process.†

Components‡	Prepress solvent	Screw press	Direct solvent
Dry matter	89.9	91.4	90.4
Ash	6.4	6.2	6.4
Crude fiber	13.6	13.5	12.4
Ether extract	0.58	3.72	1.51
Crude protein	41.4	41.0	41.4
Gossypol-free	0.05	0.04	0.30
Gossypol-total	1.13	1.02	1.04
N-solubility§	54.4	36.8	69.4
Calcium	0.15	0.16	0.15
Iron	0.011	0.010	0.009
Magnesium	0.40	0.42	0.40
Potassium	1.22	1.20	1.16
Sodium	0.04	0.04	0.04
Phosphorus	0.97	0.93	0.98
Lysine	1.71	1.59	1.76
Histidine	1.10	1.07	1.10
Arginine	4.59	4.33	4.66
Aspartic acid	3.72	3.65	3.68
Threonine	1.32	1.30	1.34
Serine	1.74	1.68	1.78
Glutamic acid	8.30	8.55	8.08
Proline	1.54	1.42	1.45
Glycine	1.70	1.69	1.69
Alanine	1.62	1.58	1.62
Valine	1.88	1.84	1.82
Methionine	0.52	0.55	0.51
Isoleucine	1.33	1.31	1.33
Leucine	2.43	2.23	2.41
Tyrosine	1.13	1.09	1.14
Phenylalanine	2.22	2.20	2.23
Cystine	0.64	0.59	0.62
Tryptophan	0.47	0.50	0.52
Copper	17.8	16.7	16.3
Manganese	20.0	21.6	20.7
Zinc	62.3	57.4	57.4
Cobalt	1.3	1.5	1.5
Biotin	0.55	0.53	0.55
Choline	2932.6	2807.2	2706.0
Folic acid	2.66	2.73	2.79
Niacin	40.3	37.8	39.2
Pantothenic acid	7.0	7.7	9.9
Pyridoxine	4.0	4.8	4.8
Riboflavin	4.0	4.2	4.4
Thiamine	3.3	9.7	7.7

† From Anon. (1982b). Adjusted means calculated by determining mean less one-half standard deviation. Exceptions: ash, fiber, and gossypol. In these cases a one-half standard deviation is added to mean value. Values for vitamins and energy are unadjusted.

‡ Components dry matter through trytophan are expressed as %, and copper through thiamine are expressed as mg/kg.

§ Nitrogen soluble in 0.02 *N* NaOH.

Lusas et al., 1977; Rosenblum, 1980). Glandless kernels can be prepared for direct consumption or for use in food as nut replacements because of the absence of gossypol. Kernels and meal fines can be flaked and extracted with hexane to produce flour. To improve oil removal, kernels are conditioned to a moisture content of 8 to 10%, heated to 71° to 82°C, and rolled into flakes. Percolation of solvent through the flakes yields high quality oil and defatted meal. With glandless cottonseed, extensive heat treatments are not needed during processing to bind gossypol in the meal or to reduce color reversion in crude oil. Binding of gossypol with proteins is not a problem with glandless products. Further, a refined oil from glandless cottonseed requires less purification processing, and as a result, less loss of neutral oil.

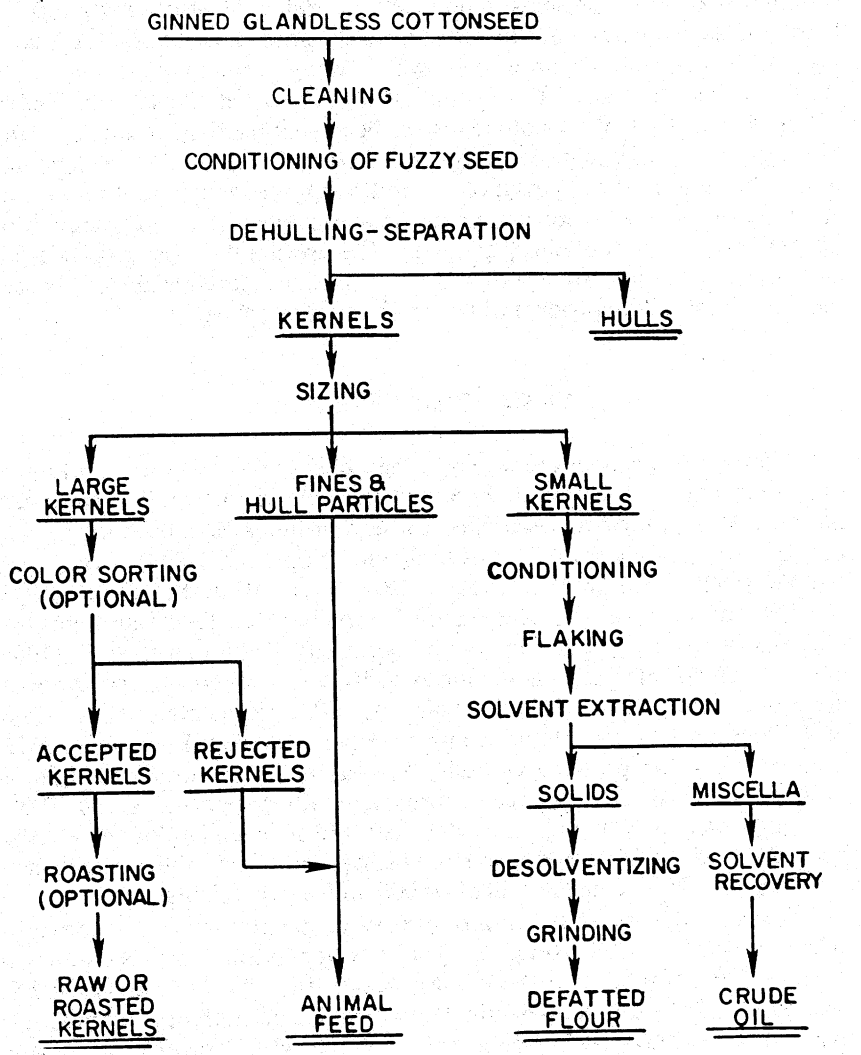


Fig. 13-8. Flow chart for production of glandless cottonseed kernels and flour (Lusas et al., 1977).

13–5.3 Phospholipid Extraction

Industrial lecithin can be fractionated as phospholipids and glycolipids after neutral lipids and protein-containing contaminants are removed (Cherry et al., 1981a; Cherry, 1983). Their purity and special properties can be improved by a number of methods, including solvent fractionation, hydrogenation, sulfonation, and ethoxylation. Lecithin, which contains both polar and nonpolar groups, has high surface activity and is reactive with both oil and protein, making it an excellent emulsifying agent in food systems.

Among common oilseeds, except for soybean, cottonseed has the highest content of phospholipids (Cherry et al., 1981a). Cottonseed phospholipids are superior to those of other oilseeds since most of the fatty acids present do not contain more than two double bonds, making them more stable to oxidation and rancidity processes (Cherry et al., 1981a; Cherry, 1983). Cottonseed phospholipids have been marketed to a small extent, however. The heat and moisture produced from the hydraulic press method of oil extraction causes gossypol to bind to phospholipids and other constituents of the meal. Solvent processing also includes heating steps that bind gossypol to the phospholipids. The availability of gossypol-free cottonseed provides an opportunity to produce food-grade cottonseed phospholipids as a byproduct of the production of edible oil.

13–5.4 Protein Extraction

Cottonseed proteins are widely recognized as potential sources of nutrients for human consumption. Because their potential as food supplements has been known for many years, cottonseed proteins have been the subject of numerous investigations. Osborne and Voorhees (1894), Jones and Csonka (1925), Fontaine et al. (1945, 1946), and Arthur and Karon (1948) showed that proteins in cottonseed meal could be extracted as albumins and globulins with water, salt, and alkaline solutions. Rossi-Fanelli et al. (1964) devised nonchromatographic methods to isolate from cottonseed a homogeneous monodispersed globulin, acalin A, with a sedimentation coefficient of 9.2 and a molecular weight of approximately 180 000. Karavaeva (1972) isolated a similar protein, globulin A, that had a molecular weight of 170 000; globulin A dissociated into two subunits of approximately 80 000 molecular weight with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Reduction and alkylation of the subunits split them further into smaller components of 40 000 molecular weights.

Based on the fact that a group of proteins in cottonseed is in compartments in aleurone grains (Fig. 13–3), a two-step extraction procedure was developed (Berardi et al., 1969; Martinez et al., 1970; Martinez, 1979). Alkaline-soluble (storage) globulins of high molecular weight precipitable at pH 7, and low molecular weight water-soluble (nonstorage) proteins precipitable at pH 4 are isolated. Alkali or salt is needed to rupture the mem-

brane structure of cottonseed protein bodies and dissolve the storage globulins. The water-soluble proteins are predominantly the functional proteins of the seed cytoplasm. The water-insoluble proteins are essentially the storage globulins of the protein bodies that contribute polypeptide fragments, amino acids, and nitrogen to the germinating seedling.

The classical procedure for isolating proteins was developed to extract the nonstorage and storage proteins together (Martinez et al., 1970). The proteins are extracted with dilute alkali at pH 10, then acidified to the isoelectric pH 5 to precipitate and collect them as an isolate. These proteins can also be selectively precipitated from the pH 10 extract at pHs 7 and 4 to prepare storage and nonstorage protein isolates, respectively.

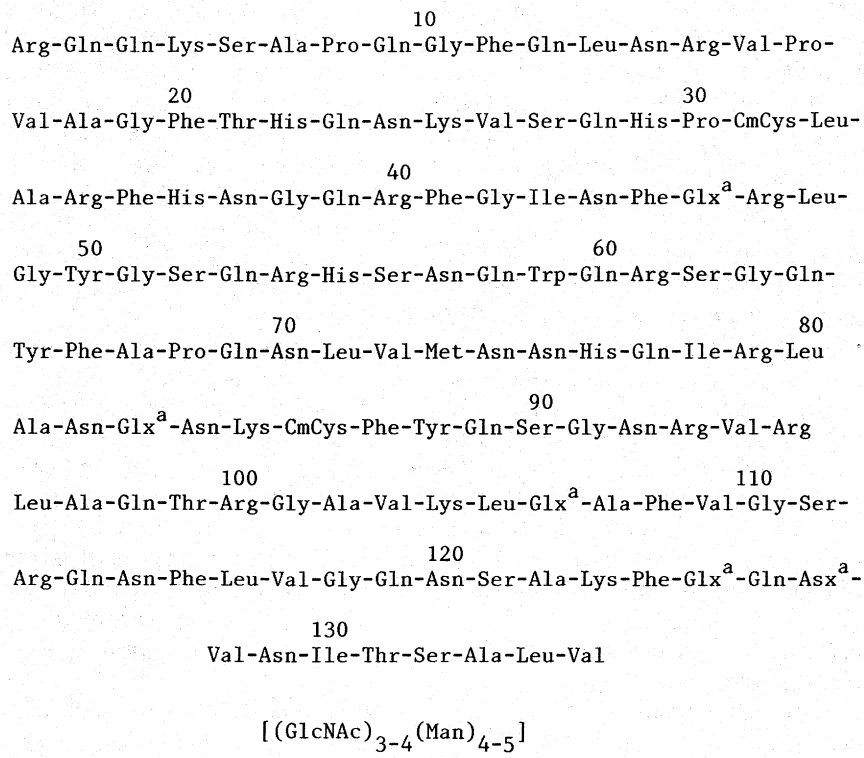
Podgornov et al. (1974) showed that a 10% NaCl extract of defatted glandless cottonseed flour could be fractionated by gel filtration chromatography into five components; two major components had molecular weights of approximately 130 000 and 300 000. Dieckert and Dieckert (1976, 1978) and Dieckert et al. (1981) similarly isolated two major storage globulins from cottonseed and labeled them acalin A and acalin B; these proteins are also known as the 7S and 12S (or 11S) globulins, respectively. Reduction of acalin A with 2-mercaptoethanol (2-Me) produced polypeptide subunits having molecular weights of approximately 49 000, 31 000, and 28 000 as determined by SDS-PAGE. The predominant subunit of acalin A has a molecular weight of 53 500 and does not exist in a disulfide-bridged subunit arrangement. A homogeneous subunit with a sedimentation coefficient of 2S and a molecular weight of 22 000 was isolated from the 7S globulin in the presence of 8 *M* urea (Ibragimov et al., 1974). Acalin B appears to have a native molecular weight of 240 000 to 250 000. Based on SDS-PAGE with 2-Me, acalin B contains polypeptides having molecular weights of 23 000, 20 000, and 15 000.

Other studies have shown that the acalin globulins consist of a large number of repetitive acidic and basic subunits bound together by disulfide bonds (Dieckert et al., 1981). The 7S globulin was shown to consist of eight polypeptides of two types which differ on the basis of carbohydrate content and partial amino acid sequences (Dieckert et al., 1981; Kuchenkova et al., 1977; Ovchinnikova et al., 1977; Redina et al., 1977). Kuchenkova et al. (1977) developed the amino acid sequence of two 7S globulin subunits (I and II; Fig. 13–9). Pronase hydrolysis of the 7S globulin allowed isolation of a glycopeptide which contained aspartic acid, glucosamine, and mannose in a ratio of 1:2:5 (Fig. 13–9; Kuchenkova et al., 1981). The carbohydrate chain has a branched structure with an N-glycoside type of oligosaccharide-protein bond.

In further studies, acalin B (11S-globulin; molecular weight of 260 000) was shown to consist of three subunits, A, B, and C, of molecular weights of 28 000, 24 000, and 17 000, respectively (Asatov et al., 1977). Since the globulin did not dissociate in 8 *M* urea and SDS, these researchers suggested that its quaternary structure may be formed by noncovalent bonds such as ionic, hydrogen, and hydrophobic interactions. The ϵ-amino group of lysine residues was shown to play an important role in the stabilization of

the 11S-globulin's quaternary structure (Shadrina et al., 1979). Asatov et al. (1978a, b) sequenced the amino acids of subunit C of the 11S globulin.

By using gel filtration chromatography, Zarins and Cherry (1981) separated the globulins contained in a 10% NaCl-extract of defatted glandless cottonseed flour into six fractions (I to VI; Fig. 13–10A and B). Column and thin-layer gel filtration methods demonstrated that these fractions had molecular weights of > 600 000, 280 000, 127 000, 63 500, 10 700, and < 2000, respectively. Fractions II and III are probably equivalent to the 12S (alcalin B) and 7S (acalin A) globulins of studies discussed above. At pH 10.5, fraction I decreased while fraction VI increased, which indicated the release of a small constituent from the large protein (Fig. 13–10A). Fractions I and VI were yellow at neutral pH; at alkaline pH, they became green-gold, which suggested that flavonol and gossypol or gossypol-like pigments were bound to these proteins (Blouin et al., 1982). Because of its large size (> 600 000), and low protein content (16.6%), fraction I was thought to be the globulins that contain fragments of aleurone grain membrane.



[a] x denotes an amide in subunit I and an acid in subunit II.

Fig. 13–9. Amino acid sequence of two 7S globulin subunits (Kuchenkova et al., 1977).

Gradient polyacrylamide gel electrophoresis under non-denaturing conditions showed that proteins in each fraction (Fig. 13–10B) were represented in the pattern of the storage protein isolate (Fig. 13–11).

The proteins in each fraction separated by gel filtration (Fig. 13–10B) were treated with SDS plus 2-Me to dissociate them to polypeptide subunits (Marshall et al., 1984). The subunits were then separated by 0.1% SDS-PAGE. Fractions I and II have similar electrophoretic patterns, but are different from those of the other four fractions (Fig. 13–12). Fractions III and IV are similar especially in the 24 000 to 63 000 molecular weight range. Seven and eight polypeptides ranging in average molecular weight from 11 750 to 63 000 were noted in the gel patterns of fractions I and II (Table 13–11). Fraction III contains eight polypeptides with average molecular weights from 15 800 to 80 200; fraction IV, which is closely associated with fraction III, has four polypeptides with average molecular weights from 26 000 to 63 000. These gels have similarities in polypeptide mobilities indicating incomplete separation, or some type association-dissociation, of fractions III and IV during gel filtration chromatography. Fractions V and VI have two and one very small polypeptides, respectively.

The amino acid compositions of the proteins in the defatted glandless cottonseed flour, nonstorage protein isolate, storage protein isolate, and fractions I, II, III, V, and VI are presented in Table 13–12 (Zarins and Cherry, 1981). The nonstorage protein isolate had a much higher amount of

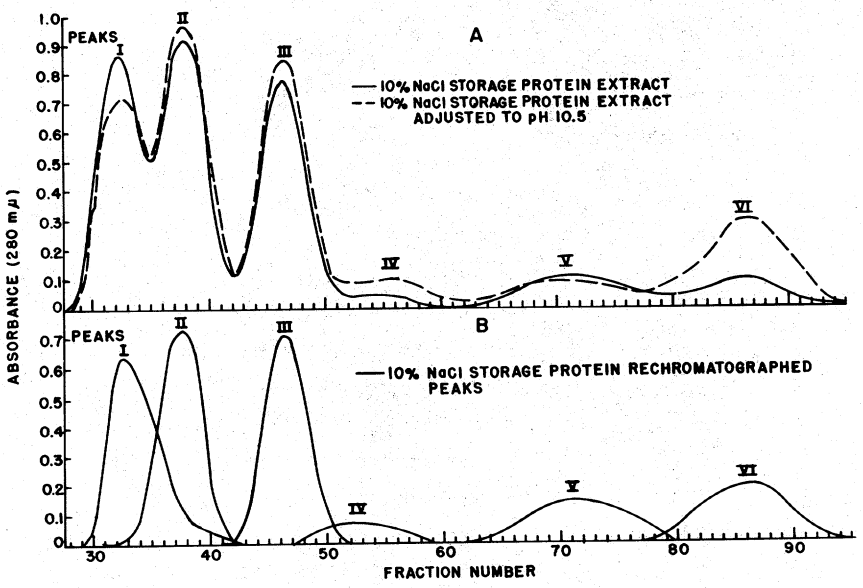


Fig. 13–10. Sephadex G-200 gel filtration chromatography of salt-soluble cottonseed protein isolate: A) protein separation of native (solid line) and alkali-treated (dashed line) salt-soluble extracts; B) composite chromatogram of the six isolated peaks (Zarins and Cherry, 1981).

Table 13–11. Molecular weights of polypeptides present in the 10% NaCl isolate and in the individual fractions I through VI (Fig. 13–10) as estimated by SDS-electrophoresis.†

Peak‡	Fraction	Molecular weight
1	Isolate	82 300
2	III	80 200
3	III	79 000
4	Isolate	75 000
5	Isolate	70 000
6	Isolate, I, II, III, IV	63 000
7	Isolate, I, II, III, IV	56 000
8	Isolate, I, II, III	41 000
9	Isolate, I, II, III, IV	35 000
10	Isolate, I, II, III, IV	26 000
11	Isolate, I, II, IV	21 200
12	Isolate, I, II, III, V	15 800
13	Isolate, II, V	11 700
14	Isolate, VI	10 000

† From Marshall et al., 1984.
‡ Corresponding to number in Fig. 13–12.

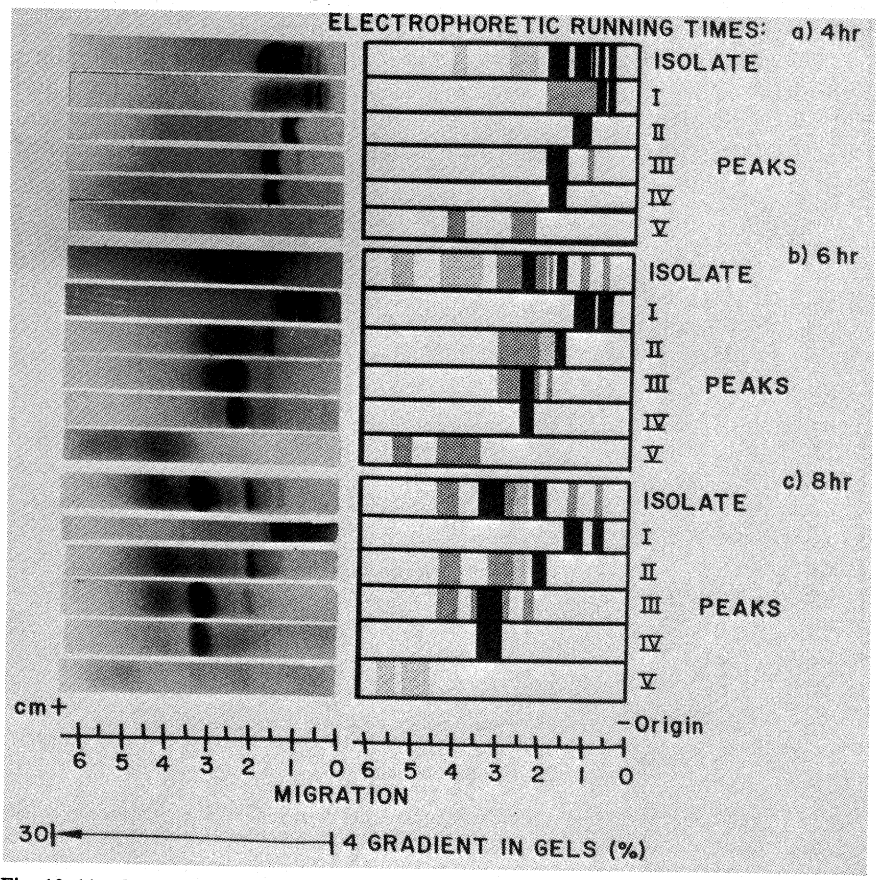
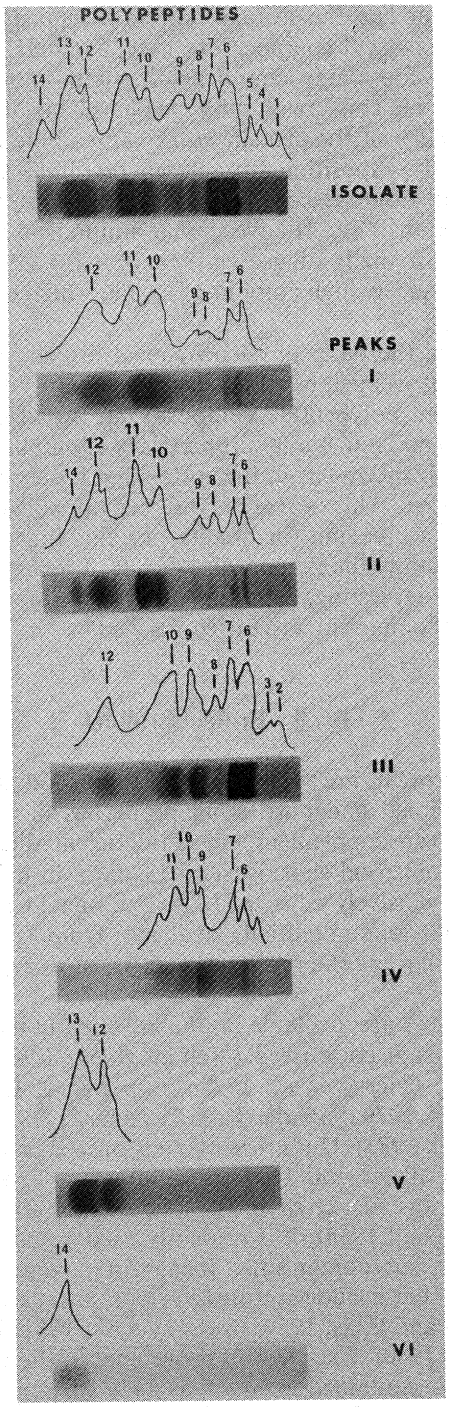


Fig. 13–11. Gradient polyacrylamide gel electrophoresis (for 4-, 6-, and 8-hr periods) of proteins in the salt-soluble isolate, and fractions obtained by gel filtration column chromatography shown in Fig. 13–10 (Zarins and Cherry, 1981).


POLYPEPTIDES
ISOLATE
PEAKS
I
II
III
IV
V
VI

lysine than the flour and the storage protein isolate. Fractions II and III have a similar amino acid composition, except that III contains very low amounts of sulfur-containing amino acids (methionine and half-cystine) and tryptophan, while II is low in tyrosine and phenylalanine. Fraction III is approximately 60% of the protein in the salt-soluble fraction of cottonseed; its amino acid composition most closely resembles those of the storage protein isolate and flour. Except for the quantities of threonine, proline, and tyrosine, fractions I and II have similar amino acid compositions, adding further support to the findings that these proteins may be alike. Fraction VI contains an extremely high amount of lysine, which explain the presence of bound pigments.

Understanding the basic physicochemical properties of cottonseed is essential to the improvement of cottonseed utilization. In particular, knowledge of protein and polypeptide compositions, structures, and contributions to functional and nutritional properties is essential to the development of technological advancements in the cottonseed industry and the maintenance of farm value. This research will provide information both for geneticists to use in breeding for quality proteins that provide high value raw agricultural commodities, and also for seed technologists to develop new and improved harvesting, handling, and processing techniques for production of quality commodities to the consumers.

13-5.5 Protein Products

Cottonseed proteins are sources of low-cost, food-grade products that can provide functional and nutritional properties in food formulations. These derivatives can supplement protein-deficient diets in areas of the world suffering from food shortages, particularly in developing countries where cotton is extensively cultivated. It is estimated that approximately one-quarter of the flour potentially available from world production of cottonseed could alleviate the protein shortages of underdeveloped nations (Gillham, 1969).

Excellent reviews are available on the production technology of edible protein products from cottonseed (Meinke, 1952; Martinez et al., 1970; Lusas et al., 1977; Cherry et al., 1978c; Experience, Inc. Report, 1978a; Berardi and Cherry, 1979a; Spadaro and Gardner, 1979; Rosenblum, 1980; Cherry and Berardi, 1983). Until recently, cottonseed protein products were used mainly as fertilizer and feed for ruminants. Proflo, a food-grade cottonseed flour, was marketed in the USA as an additive to condition bread dough (Altschul, 1958); it is presently used as a source of protein in culture media for microorganisms. Incaparina, containing mixtures of vegetable proteins that include cottonseed flour, was used as food in South America (Bressani and Elias, 1969; Bressani et al., 1961, 1969, 1980; Scrimshaw, 1980; Wise, 1980). Excellent improvement in the growth of children fed Incaparina that contained as much as 38% cottonseed flour has been reported. The flour used in these studies was processed at a mill that

ordinarily produced cottonseed meal as feed and subsequently upgraded for food according to the guidelines established by the Protein Advisory Group of WHO/FAO/UNICEF (Milner, 1965). During normal processing, heat was used to bind gossypol in the meal, making it physiologically inactive and producing a light-colored oil. Gossypol is thought to bind to the free ϵ-amino group of lysine, and possibly also to arginine and cysteine of proteins, during heating (Damaty and Hudson, 1975). Thus processing denatures proteins and causes interactions among various constituents, and lowers the nutritive value of the meal.

A primary technical objective in the processing of edible cottonseed flour, as well as products used for feeding monogastric animals, such as swine and poultry, is the reduction of the toxic impact of gossypol. Several solvents and azeotropes effectively removed gossypol, but problems with flavor, color, and adapting the processes to commercial practice discouraged their use. Pons and Eaves (1971) patented a process using aqueous acetone to lower free gossypol levels to approximately 0.01 to 0.02%. Damaty and Hudson (1975) prepared cottonseed flour with a free gossypol level of 0.01 to 0.03% and a total level of 0.20 to 0.36% by sequential extraction first with aqueous, and then dry acetone. However, during acetone extraction the sulfur-containing amino acids decompose to produce hydrogen sulfide which reacts with an acetone condensation product, mesityl oxide, to produce a "catty odor" (Alyevand et al., 1967). Canella and Sodini (1966) removed gossypol from cottonseed meal with a butanol–hydrochloride acid solution, but this process reduced the free gossypol level in the meal only to 0.070%, which was higher than the 0.045% level permitted in cottonseed products for food use.

A process described as differential settling was developed to fractionate oil, pigment glands, and hull fragments from cottonseed flour (Fig. 13–13; Vix et al., 1947, 1949, 1969, 1971; Gardner et al., 1976). The method uses the principle of mixed solvent (hexane) flotation to separate a low weight, high protein fraction from oil, gossypol, and other coarse materials. It produces edible grade cottonseed flour with free gossypol levels of less than 0.045%. The underflow fraction contains most of the gossypol and hull materials. The method has been engineered to pilot plant scale as the liquid cyclone process (LCP) (Gardner et al., 1976).

Commercial plants with stringent sanitary conditions can be developed for LCP. The edible-grade LCP flour produced at pilot plant scale at the Southern Regional Research Center meets FDA approval in the USA and the guidelines established by the Protein Advisory Group of WHO/FAO/UNICEF for food use (Experience, Inc., 1978a). A representative LCP flour contains 65 to 68% protein with solubility greater than 95% in 0.2 *N* NaOH solution, total gossypol less than 0.060%, free gossypol less than 0.045%, and lipid less than 1.000%; it also contains 2 to 3% crude fiber, 7% ash, and 3.9 g available lysine/16 g nitrogen. The product is bland in flavor, light in color, and production costs are reasonable (Experience, Inc., 1978b). On a dry weight basis, the flour qualifies as a concentrate containing approximately 70% protein. Considerable interest in the potential of LCP cottonseed products is developing throughout the world.

Methylene chloride reduces the amounts of free and total gossypol in meal extracted with hexane and the LCP underflow protein fraction of glanded cottonseed from 2.600 to 3.400% to 0.013 and 0.150%, respectively (Cherry and Gray, 1981); the free gossypol percentage is well below the 0.045% value. The cottonseed meals were pretreated in one of three ways to rupture the gossypol glands: (a) equilibrated with additional water, (b) suspended in various water–propylene glycol mixtures, or (c) mixed with an

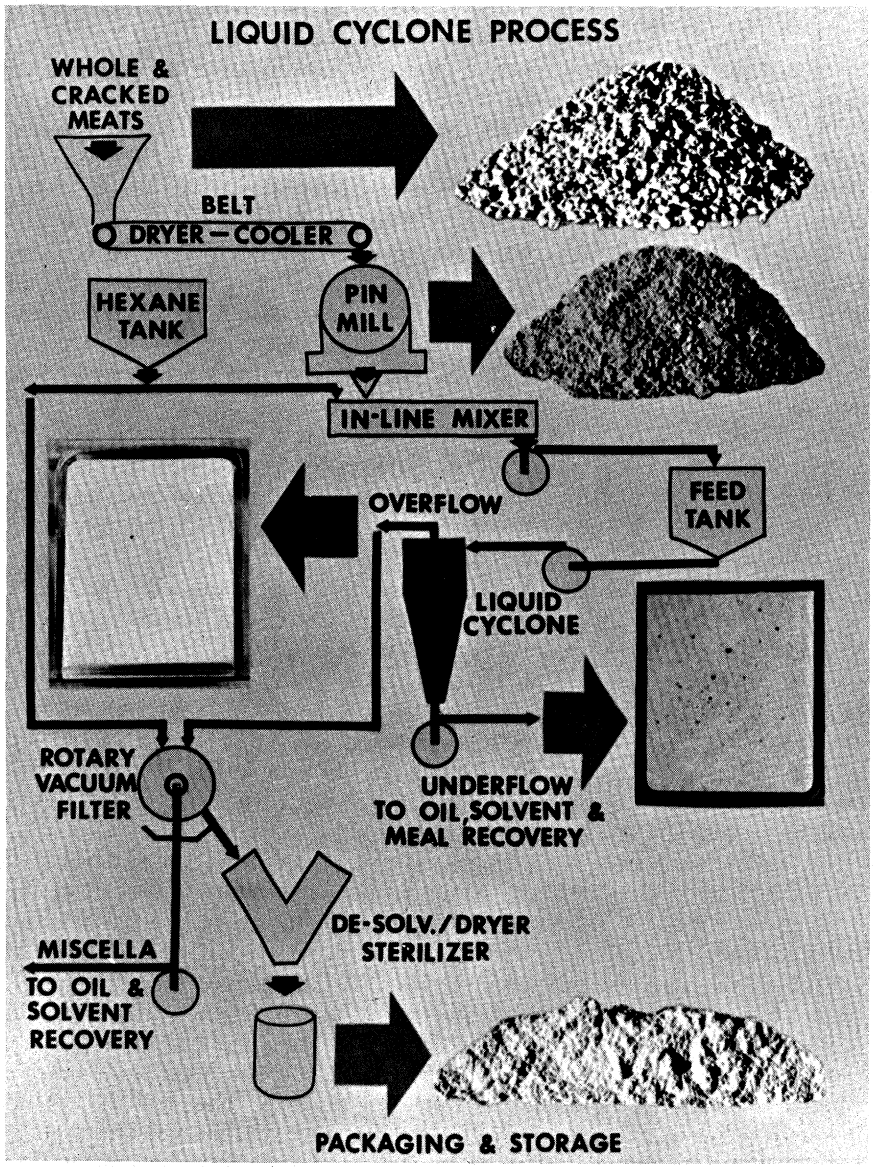


Fig. 13-13. Pilot plant LCP flowsheet (Gardner et al., 1976).

acetic acid–water–propylene glycol solution. The gossypol was then readily extracted from the meals with methylene chloride. Low levels of water and acetic acid in propylene glycol aided methylene chloride in the removal of free and total gossypol; they did not alter proximate composition, solubility, gel electrophoretic properties of proteins, amino acid content, or chemical scores of treated meals. Success with this process should improve the potential of using the underflow of the LCP as feed or food.

Edible cottonseed protein products containing as much as 60.4% protein, and as low as 0.05% free and 0.11% total gossypol can be obtained from glanded, defatted cottonseed flour by air classification (Kadan et al., 1979, 1980; Freeman et al., 1979). Optimal results are obtained if there is minimal pigment gland damage during the lipid extraction and various milling steps. The defatted material must be ground as finely as possible, without disrupting the structure of the pigment glands. Other important factors include maximum removal of hulls, a high velocity through-flow type dryer to dry flakes to 2% moisture before oil extraction, and solvent extraction of flakes to about 2% residual lipids. Defatted flour prepared this way can be fractionated into edible products containing high protein and low gossypol by multiple air classification steps.

Laboratory and pilot plant studies show that protein products from glandless cottonseed are of high quality because the difficulties attributed to gossypol are either minimized or avoided (Fig. 13–8; Lusas et al., 1977; Rosenblum, 1980). Vegetable protein products from the processes described above can be used as such in several food applications, ground into flour, or used in the production of protein concentrates and isolates (Cherry and Berardi, 1983).

Bitter-tasting, odorous, off-flavored compounds, trypsin inhibitors, aflatoxins, and flatulence sugars are usually lowered or removed in the preparation of protein concentrations from cottonseed products (Cherry and Berardi, 1983). Process variations, including water leaching at the isoelectric point, aqueous alcohol leaching, dilute salt leaching, liquid cyclone fractionation, air classification, ultrafiltration, reverse osmosis, and aqueous extraction have been developed at the experimental level to produce concentrates with 60 to 70% protein content.

All processes, except the LCP and aqueous extraction process, usually begin with defatted flours or flakes free from gossypol. The aqueous extraction process involves simultaneous separation of a pH 4.0 aqueous extract of seed into oil, solid, and aqueous phases. Acid soluble proteins, carbohydrates, oil, and other constituents are extracted, leaving the fiber and acid insoluble substances in the protein concentrate. Low molecular weight nonprecipitable proteins, sugars, and salts may be recovered from the whey by ultrafiltration and reverse osmosis (Lawhon et al., 1977a, b; 1978). Approximately 96% of the oil in seeds can be recovered by this process.

Production of cottonseed isolates with 90% or more protein involves extracting the water-insoluble polysaccharides, water-soluble sugars, and other minor constituents. These components usually remain in concentrates

(Cherry and Berardi, 1983). Several methods have been developed for producing cottonseed protein isolates (Berardi et al., 1969; Martinez et al., 1970; Lusas et al., 1977; Cherry and Berardi, 1983). These methods have been discussed earlier in this chapter. Briefly, they include the classical method, in which nonstorage and storage proteins are extracted together from flour with 0.034 *N* NaOH. The alkaline extract recovered after centrifugation is then acidified to pH 5.0 to provide a protein curd containing both storage and nonstorage proteins. The curd is collected by centrifugation and lyophilized or spray dried to form the classical protein isolate. Secondly, the selective precipitation method, the flour is mixed with 0.034 *N* NaOH, centrifuged, and the soluble fraction adjusted to pH 7.0 to selectively precipitate storage proteins. After centrifugation or filtration, the soluble portion is further adjusted to pH 4.0 to isolate the nonstorage proteins. The third method, the selective extraction, flour is suspended in water to dissolve the nonstorage proteins. After centrifugation, these proteins are precipitated and collected by centrifugation or filtration after adjusting the soluble extract to pH 4.0. The spent flour collected after the initial centrifugation step is mixed with 0.015 *N* NaOH, centrifuged, and the storage proteins are precipitated and collected at pH 7.0.

Protein, lipid, fiber, and ash are 93.4, 1.1, 0.5, and 3.4%, respectively, in a representative classical isolate; 86.2, 2.8, 0.2, and 5.9% in nonstorage protein isolates; and 98.0, 0.8, 0.1, and 1.4% in storage protein isolates. The nonstorage protein isolates are the low molecular weight components that are high in essential amino acids (Cherry and Berardi, 1983). The storage globulins with high molecular weight and low essential amino acid content are present mainly in the storage protein isolate (Table 13–12).

An improved process employing a combination of isoelectric precipitation and ultrafiltration to isolate glandless cottonseed protein was developed (Lawhon et al., 1980). Potassium hydroxide and sodium hydroxide each dissolved approximately 88% of the flour nitrogen including storage protein (SP) at pH 10, 87% at pH 9.5, and 85% at pH 9.0. Nonstorage protein (NSP), extracted prior to SP extraction with water, was separated by acid precipitation into curd and whey. The lysine-rich NSP whey was then added to SP extract and the mixture ultrafiltered to obtain a higher yield of SP isolate having a lighter color and increased lysine content.

Coprecipitated protein isolates have been prepared from blends of glandless or LCP cottonseed flour, other oilseed flours, and animal proteins (Berardi and Cherry, 1979b, 1981). More specifically, coisolates were made by coprecipitating the cottonseed proteins with soybean and peanut proteins. The coisolate method involves protein extraction with 0.034 *N* NaOH, acidification of the protein extract to pH 2.5, and adjustment of the resulting mixture to pH 5.0 to precipitate the protein curd. The recovered curd is resuspended in water, neutralized, and lyophilized. Disc-gel electrophoresis showed that some of the proteins in the extract dissociated into subunits at pH 2.5, then reassociated into their original or new protein forms as the pH was adjusted to 7.0. The coisolates contain 95% protein and accounted for more than 67% of the total nitrogen in the flour blends.

Table 13–12. Amino acid composition of glandless cottonseed flour storage proteins.†

Amino acids	Flour	Nonstorage protein isolate	Storage protein isolate	Gel filtration peaks of storage protein isolate§				
				I	II	III	V	VI
	g/100 g dry weight protein							
Aspartic acid	9.97	10.51	9.46	9.58	9.69	9.18	8.75	7.85
Glutamic acid	21.87	19.35	21.29	18.06	20.45	20.04	19.25	11.91
Alanine	4.40	6.03	3.73	4.78	5.26	3.57	4.20	4.80
Isoleucine‡	3.16	4.47	3.67	3.58	3.75	3.26	2.75	3.02
Phenylalanine‡	5.55	4.69	8.35	6.05	5.46	10.54	5.94	3.85
Threonine‡	3.73	4.65	3.02	4.00	2.63	3.04	3.38	4.19
Proline	1.19	4.78	1.08	3.51	1.26	1.48	3.60	0.80
Valine‡	4.67	2.99	4.69	4.00	4.86	5.49	3.76	4.12
Leucine‡	6.03	4.33	6.34	6.80	6.72	6.36	4.99	5.45
Histidine‡	3.12	2.48	3.10	2.63	2.97	3.71	3.29	2.49
Arginine‡	12.78	8.53	12.67	12.13	15.99	12.46	9.67	23.06
Serine	4.88	4.14	5.00	6.21	4.33	5.37	8.31	7.31
Glycine	4.61	4.52	4.19	5.68	5.38	3.85	5.91	6.66
Methionine‡	0.67	1.59	1.45	1.14	1.22	0.21	0.63	1.46
Tyrosine	2.09	3.15	3.88	4.27	0.66	4.06	4.11	2.56
Lysine‡	4.86	8.09	3.13	2.10	2.27	3.12	2.65	8.43
Half-cystine	2.22	3.25	1.53	--	2.03	0.57	--	--
Tryptophan	2.05	--	1.16	--	2.49	1.31	--	0.07
Ammonia	2.14	2.48	2.27	5.29	3.43	2.18	6.76	2.01

† From Zarins and Cherry (1981).
‡ Essential amino acids.
§ Corresponding to numbers in Fig. 13–10.

13–6 UTILIZATION

13–6.1 Planting Seed Properties

The processes that result in germination are initiated by the uptake of water by the seed. When plotted against time, under laboratory conditions, the water content of delinted cottonseed increases sigmoidally during the first few hours (Dewez, 1964; Krieg and Bartee, 1975; Leffler and Williams, 1983). After an initial rapid increase in the rate of water uptake, further absorption occurs, but at a reduced rate (Dewez, 1964; Krieg and Bartee, 1975). This rate change indicates not only that water uptake is sustained, but also that it is probably regulated, either osmotically or metabolically. With many seeds, a hard-seed character can severely restrict the imbibitional hydration of cottonseed. In seed with this characteristic, the chalazal end is highly compacted and is effectively plugged by substances that are impermeable to water (Christiansen and Moore, 1959). This hard-seededness can be broken by subjecting the seed to a hot (85°C) water treatment (Walhood, 1956). The vast majority of cottonseed are not hard-seeded, however, so this character normally presents little problem in seedlots of cultivars destined for use as planting seed.

Upon hydration of the embryonic tissues, an environment is created within the embryo in which rapid metabolic activity can resume. The principal storage reserves in the cotyledons of the cotton embryo are proteins and oils; the initial phases of germinative metabolism are dominated by the mobilization of the oil reserves. Lipase action upon the glycerides of the embryo produces free fatty acids, low levels of which are detected during the early stages of germination (Christiansen and Moore, 1961; St. Angelo and Altschul, 1964). Only low levels of free fatty acids accumulate because of the rapid development of high amounts of glyoxylate cycle activity, through which carbohydrates are formed. These carbohydrates are utilized as substrates for the respiratory production of energy; they are also translocated, along with amino acids produced by proteolysis, to the developing axis.

Once germination has been initiated, sustained vigorous growth by the developing axis becomes the next critical period in the establishment of a cotton plant. Axis growth rates are influenced by the initial quality of the seed, the metabolism of the cotyledonary storage reserves, and the moisture and temperature regime of the seedbed. Selection of the seed within a seedlot that have an optimum density results in the maximum expression of axis growth (Leffler and Williams, 1983). Under favorable temperature conditions, radicle growth between 3 and 12 days was significantly correlated with the depletion of both lipid and non-lipid cotyledonary reserves (Krieg and Carroll, 1978). When temperatures were less than optimum, however, lipid use was correlated with radicle growth only between 3 and 6 days, while non-lipid utilization assumed primary importance between 6 and 12 days. Except under extreme moisture and temperature conditions, the axis growth rate is generally proportional to the seedbed temperature. Guinn (1965) reported that low temperatures suppressed root growth more than hypocotyl growth. Wanjura et al. (1967) identified a consistent relationship between the initial emergence of cotton seedlings and the cumulative number of hours, since planting, that soil temperature exceeded 18°C. Their data suggested the presence of a low temperature threshold for axis growth.

Although it is not the final step in the germination sequence, the emergence of the seedling is a decisive factor in the process of stand establishment. While the initiation of germination and rate of axis growth are fundamental processes associated with emergence, they are not entirely responsible for the success or failure of emergence. Among the other factors that often negatively affect the emergence of seedlings is the formation of a crust on the soil. The characteristics of the soil primarily related to formation of crust are the texture and organic matter content (Russell, 1957; Wilkes and Corley, 1968). The after-effects of a rain between planting and emergence can be detrimental to the establishment of a stand (Wanjura and Minton, 1981). Crusting poses a more serious problem in soils that are light-textured and low in organic matter (Wilkes and Corley, 1968). Soil crusting

may be avoided by the selection of planting dates to minimize the likelihood of adverse weather and by the adjustment of the planting depth. Currently, there are two main ways to overcome soil-crusting problems: (a) the use of high-quality seed that emerge rapidly, before a crust fully forms, and (b) the use of implements, such as the rotary hoe, to destroy the crusted layer so that seed that germinate slowly can survive and produce seedlings that will emerge. Although seeding at excessive rates is practiced in some areas to overcome soil crusting problems, this practice introduces the potential problem of excessive stands when growing conditions are optimum.

Because of the importance of the germination capacity of a seedlot as a production resource, many attempts have been made to develop assays to estimate this quality. While each procedure undoubtedly measures certain aspects of seed quality, each also possesses deficiencies in the accurate prediction of the actual field performance of the seedlot (Delouche, 1981). The most often observed contrast between these laboratory procedures and field performance is that the former overestimates the latter; this contrast becomes more pronounced as field conditions become increasingly severe.

A commonly accepted reason for this divergence of results is that the standard germination test (AOSA, 1978) is conducted under highly favorable conditions that are not realized in field plantings. The temperature regimes accepted for the standard test are either (a) alternating cycles of 20° and 30°C, or (b) a constant 30°C. Because field plantings are often made before soil temperatures remain consistently above 20°C, even the alternating-temperature regime provides an unusually favorable environment for germination. This differential performance in these environments is indicative of the fact that the individual seed within a seedlot do not fall into discrete classes. Conversely, the field expression of germination potential by a given seedlot forms a continuous distribution that ranges from zero to a relatively high value. Whereas germination per se may, in fact, fall into discrete classes, field peformance is dictated by the success or failure of seedling emergence and survival. Survival is predicated upon an expression of vigorous axis growth under adverse environments.

Two vigor assays, the tetrazolium test and the Texas cool test, have been used in the evaluations of planting cottonseed quality (Delouche, 1981). The tetrazolium vigor test consists of a subjective interpretation of the tetrazolium viability test (Baskin, 1981c). With this test, seeds are classified as either non-germinable or low-, medium-, or high-vigor on the basis of the intensity and distribution of red stain and tissue turgidity after they have been immersed in a solution of 2,3,5-triphenyl tetrazolium chloride. Because of the subjective nature of this evaluation, however, interpretation and scoring of results are both time-consuming and difficult to make. Therefore, this quality test will probably not gain widespread adoption (Baskin, 1979). The Texas cool test, on the other hand, utilizes the same procedure as does the standard germination test, but it is conducted at 18°C and covers a period of either 6 days, for acid-delinted seeds, or 7 days, for

either machine-delinted or gin-run seeds. Because of the similarities between the cool test and the standard test, the cool test will likely gain wider acceptance (Baskin, 1979).

Wanjura et al. (1969) measured the productivity of cotton plants that had emerged at different times; they found that the vast majority of the yield came from the first plants to emerge. In comparisons of fully and partially developed cottonseed, Ferguson and Turner (1971) found that the degree of development was positively associated with both emergence and early seedling vigor. A favorable association between the rate of initiation of germination and the rate of early seedling growth has been attributed to seed density selection (Tupper et al., 1970, 1971; Krieg and Bartee, 1975; Krieg and Carroll, 1978; King and Lamkin, 1979). More recently, Leffler and Williams (1983) found that the growth advantage developed by the earliest germinating seedlings increased throughout the first month of seedling growth. Similarly, both Turner and Ferguson (1972) and Minton and Supak (1980) reported that seed quality influenced yield in ways that extended beyond the direct influence on stand. Turner and Ferguson (1972) detected significant differences in seedling populations due to the degree of seed development; they then thinned plots to a uniform population and measured the yield from these plots. Those plots that had initially contained the highest population subsequently produced the highest yield, even after the plots had been adjusted to a common seedling population. Similarly, Leffler et al. (1978) identified significant effects of seed quality on both stand and yield; seed quality differences were associated with the portion of the season in which the seeds developed. Using seeds of selected densities, Minton and Supak (1980) identified influences of the seedlots on stand, disease susceptibility, and yield.

The growth of a cotton crop is continuously influenced by all the events that have affected the preceding phases of crop development. Plant populations are affected by seed quality and seedling growth rates. In addition, plant growth is influenced by plant density per unit area. Young seedlings growing in thick stands tend to grow taller than those in thinner stands. Light penetration into the canopy in thinner stands may cause the plants to effectively initiate reproductive development sooner than those in thicker stands. By altering both inter-row and intra-row spacings to maintain a constant total population density, Walhood and Johnson (1976) created significantly different competitive environments for seedling growth. Uniform spacing of plants ultimately resulted in yields 40% greater than those produced by plants grown in the "standard" planting pattern.

13-6.2 Food Functional Properties

Research has shown that cottonseed protein derivatives compete well with other vegetable protein products in food formulations (Meinke, 1952; Martinez et al., 1970; Lawhon and Cater, 1971; Lusas et al., 1977; El-Sayed et al., 1978a, b; Experience, Inc., 1978a; Spadaro and Gardner, 1979; Berardi and Cherry, 1979a; Terrell et al., 1979; Rosenblum, 1980; Manak et al., 1980; Cherry, 1983; Cherry and Berardi, 1983). Numerous potential

Table 13–13. Potential uses of cottonseed protein products in food and industrial products.†

Product	Function	Effect
Meat		
Ground beef; sausages (frankfurters and wieners); frozen meat patties; canned meats with sauces and casseroles; simulated meats	Extender and substitute as flours, concentrates, isolates, and texturized proteins; emulsifier; binder; fat and water absorption; thickening agents	Reduces stickiness, increases juiciness; improves texture, firmness, and snappiness; enhances oil and water absorptivity
Baked goods		
White bread; health and dietetic breads; donuts; biscuits; cookies; cakes; waffles, pancake mixes, crackers; milk replacers	Protein fortifier as flour concentrates, isolates and extrudates; color; flow control; dough conditioner; synergistic properties of composite proteins	Nutritional supplements; specialty products for dietetics; improves yellow color; maintains size uniformity; enhances texture
Pasta		
Macaroni, spaghetti and noodles	Protein fortifier as flours, concentrates and isolates; meat substitutes; color	Nutritional supplements; improves yellow color
Tortillas and tacos	Protein fortifier as flours, concentrates, and isolates	Nutritional supplements
Snack foods (potato chips, pretzels, etc.)	Protein fortifier as flours, concentrates and isolates; texture	Nutritional supplements; improves textural properties
Dairy		
Caseinate, albumin, and nonfat milk solids replacers; cheese substitutes; soft frozen desserts, puddings, ice cream and yogurts	Flours, concentrates, and isolates as milk replacers; rennin activity; substitutes and extenders; texture, body, color	Nutritional supplements; improves texture, body and color
Breakfast foods; diet and health foods	Protein fortifiers	Nutritional supplements
Beverages	Protein fortifiers; acid pH solubility	Nutritional supplements
Confections	Substitutes and extenders	Cocoa and chocolate substitutes
Industrial uses—calf milk replacers; pet foods; plywood glues	Substitutes; extrudates; protein fortifiers; extenders	Nutritional supplements; viscosity

† From Experience Incorporated (1978a).

uses were developed for cottonseed proteins in foods (Table 13–13). Functional properties, flavor, and color of cottonseed products meet consumer acceptance.

Cottonseed flour in water can be emulsified with oil to viscosities that simulate salad dressing and mayonnaise products (Cherry et al., 1978c, 1979a; Cherry and McWatters, 1981; McWatters and Cherry, 1981). Suspensions that are low in soluble protein (especially at the isoelectric point) do not form emulsions (Fig. 13–14). Mayonnaise-like products are formed with suspensions adjusted to low acidic or alkaline pH, where most storage proteins are soluble. Excellent foam volumes with good stabilities are obtained from protein suspensions adjusted to pHs between 3.0 and 3.5 (Fig. 13–15; Cherry et al., 1979a; Cherry and McWatters, 1981). The

volumes of these foams are greater than those of egg white solids at comparable protein concentration and pH. Storage proteins produce greater foam volumes than the nonstorage fraction. The excellent whippability, and emulsifiability of cottonseed isolates at low pH may be partially attributed to many of the proteins being acid-dissociated. Between pH 4.5 and 8.0, the egg white standard performs better than the cottonseed proteins. Isolates from cottonseed flours produce cream-colored foams and emulsions at acid pH, while they are tan at alkaline pH. The capacity of cottonseed products to perform functionally in these ways suggests that they may be used to improve the protein content of food formulations such as frozen desserts, whipped toppings, and mayonnaise or salad dressing-type products.

Water and oil absorptivity of cottonseed flours are not appreciably affected by pH changes. Absorption values of 2.5 g of water or 1.5 g of oil per g of flour are noted (Berardi and Cherry, 1979a). Nonstorage and storage protein isolates respond differently in water and oil absorption tests when their pH vlaues are between 3 and 8. The storage protein isolate absorbs

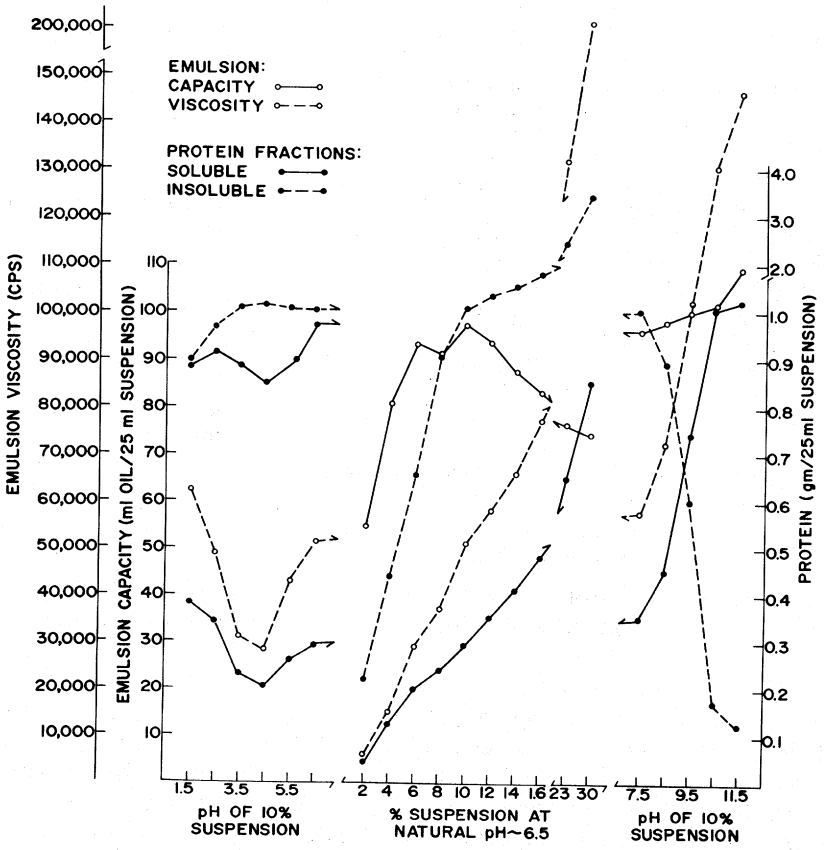


Fig. 13-14. Emulsion properties and content of soluble constituents related to pH and percentage of glandless cottonseed flour in aqueous suspensions (Cherry et al., 1978c).

more oil than water between pHs 3 and 5, but the reverse is true between pHs 5 and 8. The nonstorage proteins absorb more water than oil at all pHs. An example of performance differences among isolates is shown at neutral pH: 1 g of storage protein absorbs 1 g of water and 0.95 g of oil, and 1 g of nonstorage protein absorbs 3 g of water and 2 g of oil at about 25°C. Water binding capacity reflects the ability of flours and isolates to be incorporated into aqueous food formulations. Oil binding capacity determines the performance of cottonseed protein derivatives as meat analogs or extenders. Differences in water and oil absorptivity of cottonseed protein products are probably due to compositional variations (molecular weight, configuration, amino acid content, and solubility properties).

Minor structural changes of cottonseed proteins, through acylation with various acid anhydrides including acetic, dimethylglutaric, maleic, and succinic anhydrides, can improve extractability of proteins and certain

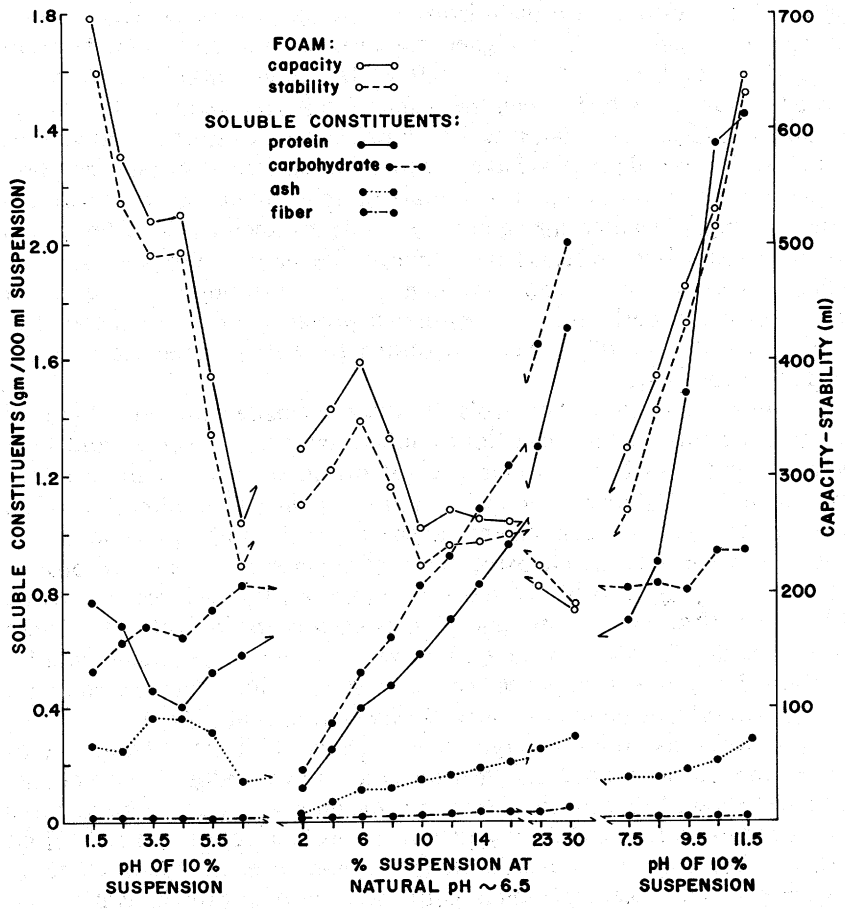


Fig. 13-15. Foam properties and content of soluble constituents related to pH and percentage of glandless cottonseed flour in aqueous suspensions (Cherry and McWatters, 1981).

functional properties of cottonseed products required for food application (Cherry and McWatters, 1981; Choi et al., 1981, 1982a). In the production of isolates, bulk density was decreased, which resulted in fluffy products. Succinylated glandless cottonseed protein isolates showed markedly improved characteristics (bulk density, whitening capacity, cream separation, and oil retention capacity) as coffee whiteners when compared to nonsuccinylated products (Choi et al., 1982b). Replacement of approximately 50% of the sodium caseinate with succinylated cottonseed protein isolate did not affect the quality of whiteners relative to that of 100% sodium caseinate-based whitener.

Spinning and thermoplastic extrusion processes further expand the potential utilization of cottonseed protein products as meat extenders (Taranto et al., 1975, 1978a, b; Cegla et al., 1978; Rhee et al., 1981). Berardi and Cherry (1980) and Cherry and Berardi (1982) developed a simple heat-stir procedure to produce texturized products and self-sustaining gels from cottonseed protein isolates. Storage protein isolates produce self-sustaining gels in 6% or higher suspensions adjusted to pHs between 3.5 and 4.0 or between 9.5 and 10.0 while being heat-stirred to 90°C. Texturized products form when the suspension is adjusted to pHs between 4.5 and 9.0. Classical isolates, which contain both nonstorage and storage proteins. Texturized proteins have a sponge-like texture; taste panelists have suspension prior to heating. The storage protein isolate absorbs up to 2.5 times its weight in water during heating, while producing gels or texturized proteins. Texturized proteins have a sponge-like texture; tase panelists have judged them to have properties like cooked hamburger in tests for chewiness and mouth feel. The texturized proteins can be dehydrated to provide a stable product, then rehydrated to their original forms when needed for food use.

Compared to all-meat controls, frankfurters made with 5% cottonseed proteins (glandless cottonseed flour and storage protein isolate, and LCP flour) generally had comparable pH values, cured color, skin firmness, texture, and desirability as judged by sensory panels (Terrell et al., 1981). At 10 to 15% levels of replacement, however, panels judged these properties to be less desirable. At all percentages of replacements, storage protein isolates were judged to be the most desirable. Further studies comparing 10% flour, 10% concentrate, and 10% isolate-extended beef patties with all-beef patties showed that the seed protein ingredients retarded oxidative rancidity development, improved cooked yields, retained less fat, and were not significantly different in sensory quality (Ziprin et al., 1981).

Physical tests (mix viscosity, overrun, penetrability, and Hunter color values) and sensory evaluations (texture, color, flavor, odor, and overall acceptance) showed that up to 40% of nonfat milk solids could be replaced with glandless cottonseed flour and storage protein isolate and LCP flour with satisfactory results in frozen desserts (Simmons et al., 1980). Of all ingredients tested, except glandless cottonseed storage protein isolate, acceptability and functionality in the desserts tended to fall at or above 40%

substitution. The glandless storage protein isolate might successfully replace up to 80% of the nonfat milk solids in frozen desserts. Protein isolates produced from glandless cottonseed flour by ultrafiltration with an industrial membrane system had functional properties equal or superior to those of a commercial soybean isolate (Manak et al., 1980). Incorporation of whey proteins into the membrane-separated isolates markedly enhanced their functionality. Cottonseed protein products have reasonably bland flavors. Flours and isolates of glandless cottonseed are lighter colored than those of corresponding LCP products. The color of all cottonseed protein derivatives is pH dependent; it is white in the acid pH range, but yellow to green at alkaline pH (Blouin et al., 1982).

Vegetable protein products of glandless and LCP cottonseed have the potential of becoming useful food ingredients. These products are (a) free from foreign matter, microorganisms, and toxic substances; (b) bland in flavor and light colored; (c) stable under most processing conditions; (d) processed at competitive costs; (e) nutritious; (f) functionally desirable; (g) available in various forms suitable for different food uses; (h) useful as additives in traditional as well as new food formulations; and (i) with unique marker characteristics that are distinguishable and readily detectable in food blends.

13-6.3 Other Uses

The seed fuzz that are not removed during the ginning operation are known as linters (Van Wyck, 1948). During preparation of cottonseed for processing into edible oil and flour, and animal feeds, the linters are removed from the seeds. Three major types of linters are recovered: first-cuts, 0.15 to 0.38 kg/10 kg of seeds; second cuts, 0.90 to 1.05 kg/10 kg of seeds; and mill-runs, 0.35 to 1.00 kg/10 kg of seeds. First-cut linters are used in surgical, paper, and packing products; second-cut linters, in chemical cellulose for preparation of regenerated fibers, films, lacquers, explosives, plastics, and paper; and mill-run linters in chemical cellulose and padding products (Dunning, 1948; Glade and Ghetti, 1979).

Cottonseed hulls are used primarily as roughage in livestock feed products, and have been used as fuel for oil mills, insulation material, soil conditioner, thickener for oil well drilling muds, filler for phenolic plastics to improve impact strength, cellulose for regenerated fiber production, and a source of xylose and furfural (Dunning, 1948). Cottonseed raffinose has been used in certain culture media (Dunning, 1948).

In addition to its use as a source of feed and vegetable protein products for food, cottonseed meal is a source of technical proteins (Bailey, 1948), and it has been used as an adhesive (Arthur, 1950; Arthur and Hogan, 1953a, b; Hoffman and Arthur, 1952; Hogan and Arthur, 1951a, b, 1952). Its proteins have been extruded to form fibrous material for use by the textile industry (Arthur et al., 1949).

13-7 NUTRITION

Guidelines for edible, high nutritional value cottonseed protein flours and related products were recently updated (Milner, 1980). Cottonseed, to be suitable for preparing edible, high nutritional value products, should have less than 1.0% foreign matter and 10.0% moisture, not more than 1.8% free fatty acids in the oil, and fewer than 5.0% discolored kernels. The total gossypol should not be subjected to conditions before processing that cause gossypol to bind to lysine. During storage, cottonseed should contain less than 12% moisture and stored below 50°C. The kernels should be free from insect damage and bacterial and mold contamination. Aflatoxin-free, high-quality cottonseed should be cleaned, delinted, and dehulled to produce meats. The meats should be suitably prepared for oil recovery employing either continuous screw-pressing, pre-press solvent extraction, or direct solvent extraction. Minimization of heat, moisture, and processing time is essential to maintain protein quality and to obtain a free gossypol content of not more than 0.045% in the meal. Processing methods must be engineered to provide flours with high nitrogen solubility, low lipid content, not more than 60 ppm hexane residue, less than 30 to 50 ppb total aflatoxins, and less than 0.2 ppm arsenic. In the USA, new installations or satellite plants operating under stringent sanitary conditions for food production appear to be the only avenue for preparing edible-grade cottonseed flours.

The nutritional value for cottonseed oil primarily provides food energy, essential fatty acids, particularly linoleic acid, and vitamin E (Cherry and Berardi, 1983). Cottonseed meal is used principally as a feed that provides proteins to ruminant livestock (Smith, 1970). Table 13-10 presents the compositions of cottonseed meals prepared by different processes. Extensive research, supported by practical experience, suggests that cottonseed meal, if heated to inactivate physiologically active free gossypol, can be a major oilseed supplemental protein source in nonruminant rations (Anon., 1966). Increasing amounts of currently produced commercial meals are being utilized for nonruminant rations, supplemented with iron salt (ferrous sulfate) to inactivate free gossypol.

When treated with ferrous sulfate, glanded cottonseed kernels containing 0.75% free gossypol can be safely used to replace 50% and 17% of the protein in rat and pig diets, respectively (Clawson et al., 1975a, b). Glandless cottonseed kernels fed as raw, cooked, or roasted ground flours at 20% of the diet caused no significant detrimental effects to the number of litters born, litter size, or weights of the young of sexually mature rats or their offspring (Reber and Pyhe, 1980; Reber, 1981). Growth and food consumption were similar among rat offspring in all treatments.

Food-grade cottonseed protein products were successfully used to rehabilitate malnourished infants in Central and South America (Bressani et al., 1961; Behar and Bressani, 1966; Bressani and Elias, 1969; Milner, 1969; Graham et al., 1969, 1970; Bressani et al., 1980; Schrimshaw, 1980; and Wise, 1980). In India, Srikantia and Sahgal (1968) found successful use of

cottonseed flour in reducing protein caloric malnutrition when the free gossypol content was low. For example, Behar and Bressani (1966) and Bressani et al. (1980) showed that the nutritional value of a product identified as Incaperina Mixture No. 9 was of high quality and supported growth of children and infants.

Alford et al. (1977) and Thomas et al. (1979) demonstrated that cottonseed proteins maintained nitrogen balance in women when fed at the higher levels of protein intake consumed in the USA. Alford and Onley (1978) found that the minimum cottonseed protein required to maintain nitrogen balance in women was 0.106 g N per kg of body weight. Using the factor for estimating protein from nitrogen results in 0.66 g of protein per kg of body weight. This factor is greater than the FAO recommendation of 0.52 g for a safe level of protein intake required for adult women. Glandless cottonseed protein and LCP cottonseed values for the minimum required intakes agreed closely.

Careful processing of cottonseed products is important for the maintenance of protein quality (Lawhon and Cater, 1971; Harden and Yang, 1975; Olsen, 1973). Martinez and Hopkins (1975) reported on the calculated nutritive quality and protein efficiency ratio (PER) of protein isolates prepared by classical, selective precipitation, or selective extraction processes from glandless cottonseed and LCP flours. Isoleucine is the first limiting essential amino acid; threonine, lysine, and methionine are present in amounts that can be limiting in certain products.

Rats fed a diet containing casein had greater serum and high-density lipoprotein (HDL)-cholesterol concentrations as well as increased lecithin:cholesterol acyltransferosa activities than those fed a diet containing glandless cottonseed flour (Park and Liepa, 1982). Animals fed an arginine-supplemented casein diet to make its arginine-to-lysine ratio similar to that found in cottonseed protein showed a decrease in both serum and HDL-cholesterol when compared to the casein control group. The addition of lysine to the cottonseed protein diet to duplicate the arginine-to-lysine ratio of casein caused an increase in the same two cholesterol fractions. The hypothesis that a plant protein is hypocholesterolemic because of its ratio of arginine-to-lysine is supported by the results obtained with the feeding of cottonseed protein diets.

13–8 CONCLUSIONS

Cottonseed quality is the cumulative product of the plant's genetics, the physiology of the plant upon which development occurs, the environment to which the seed is subjected between boll opening and harvest, and the effects of the harvest and post-harvest practices. Although the post-maturation and pre-harvest environment is not usually subject to management influences, several factors can be manipulated to affect the physiological competence of the parental canopy and the quality–preservation efficiency of the harvesting and processing procedures. Since the quality and

the quantity of the seed crop often appears to be inversely related, production of a cottonseed crop might reasonably be expected to incorporate different management practices than those for the production of fiber.

Interest in the potential of cottonseeds as a source of edible vegetable protein products has been stimulated by an increased understanding of constituents; e.g., protein, oil, etc., physicochemical, functional, and nutritional characteristics of food products. Further expansion in the processing and utilization of cottonseed products may be constrained by economic conditions rather than by limitations in functionality, nutritional quality, or consumer acceptability. Should changes occur to improve the competitive position of cottonseed, the potential contributions of their products may be realized.

REFERENCES

Adams, R., T. A. Geissman, and J. D. Edwards. 1960. Gossypol, a pigment of cottonseed. Chem. Rev. 60:555–574.

Alford, B. B., and K. Onley. 1978. The minimum cottonseed protein required for nitrogen balance in women. J. Nutr. 108:506–513.

----, S. Kim, and K. Onley. 1977. Nitrogen balance of women consuming cottonseed protein. J. Am. Oil Chem. Soc. 54:71–74.

Allan, E., A. R. Johnson, A. C. Fogerty, J. A. Pearson, and F. S. Shenstone. 1967. Inhibition by cyclopropene fatty acids of the desaturation of stearic acid in hen liver. Lipids 2:419–423.

Altschul, A. M. 1948. Biological processes of the cottonseed. p. 157–212. *In* A. E. Bailey (ed.) Cottonseed and cottonseed products. Interscience Publishers, Inc., N.Y.

----. 1958. Processed plant protein feedstuffs. Academic Press, N.Y.

Alyevand, F., G. Coleman, and D. R. Haisman. 1967. Catty odours in food: the reaction between mesityl oxide and sulfur compounds in food stuffs. Chim. Ind. (Paris) 37:1563–1564.

Anon. 1950. Cottonseed and its products. p. 16–17. 8th ed. Natl. Cottonseed Prod. Assoc. Inc., Memphis, Tenn.

----. 1966. Proceedings of a Conference on inactivation of gossypol with mineral salts. New Orleans, La. Natl. Cottonseed Prod. Assoc. Inc., Memphis, Tenn.

----. 1978a. Cottonseed and its products. p. 12–13. 8th ed. Natl. Cottonseed Prod. Assoc. Inc., Memphis, Tenn.

----. 1978b. Growth regulators for cotton. Agric. Res. 27:10–12.

----. 1979. Pix. BASF Aktiengesellschaft, D-6700 Ludwigshafen, Federal Republic of Germany.

----. 1980. Cottonseed quality goals. Natl. Cottonseed Prod. Assoc. Inc., Memphis, Tenn.

----. 1982a. World forecast: record oilseed production. J. Am. Oil Chem. Soc. 59:752A–766A.

----. 1982b. Cottonseed meal. Natl. Cottonseed Prod. Assoc. Inc., Memphis, Tenn.

Arthur, J. C. 1950. Stabilization of viscous dispersions of cottonseed proteins. U.S. Patent 2,531,383.

----, and J. T. Hogan. 1953a. Cottonseed meal glues of low alkalinity. U.S. Patent 2,646,408.

----, and ----. 1953b. Cottonseed meal glue. U.S. Patent 2,662,023.

----, and M. K. Karon. 1948. Preparation and properties of cottonseed protein dispersions. J. Am. Oil Chem. Soc. 25:99–102.

----, ----, A. F. Pomes, and A. M. Altschul. 1949. U.S. Patent 2,462,933.

Asatov, S. I., E. G. Yadgarov, T. S. Yunusov, and P. Kh. Yuldashev. 1978a. The globulins of cottonseeds. XVII. Primary structure of the carbohydrate containing submit C of the 11 S globulin. Khim. Prir. Soedin 4:541–542.

----, T. S. Yunusov, and P. Kh. Yuldashev. 1978b. The globulins of cottonseeds. XV. Primary structure of a glycopeptide of submit C 11 of the 11 S globulin. Khim. Prir. Soedin. 4:539–540.

----, ----, and ----. 1977. A study of the structure of the 11 S globulin from cottonseeds. XIV. Tryptic peptides of submit C. Khim. Prir. Soedin. 2:272–273.

Ashworth, L. J., and J. L. McMeans. 1966. Association of *Aspergillus flavus* and aflatoxins with a greenish yellow fluorescence of cottonseed. Phytopathology 56:1104–1105.

Association of Official Seed Analysts. 1978. Rules for testing seed. J. Seed Technol. 3:1–126.

Bailey, A. E. (ed.). 1948. Cottonseed and cottonseed products. Wiley, N.Y.

Baskin, C. C. 1979. Germination test is not enough. p. 53–54. Proc. Beltwide Cott. Prod. Mech. Conf., Phoenix, Ariz.

----. 1981a. Storage of seed cotton. p. 310–311. *In* J. M. Brown (ed.) Proc. Beltwide Cott. Prod. Mech. Conf., New Orleans, La.

----. 1981b. Storage of bulk cottonseed. p. 308–310. *In* J. M. Brown (ed.) Proc. Beltwide Cott. Prod. Res. Conf., New Orleans, La., Natl. Cotton Council, Memphis, Tenn.

----. 1981c. Tetrazolium evaluation of cottonseed. p. 312. *In* J. M. Brown (ed.) Proc. Beltwide Cott. Prod. Res. Conf., New Orleans, La., Natl. Cotton Council, Memphis, Tenn.

Behar, M., and R. Bressani. 1966. Experience in development of incaparina for the pre-school child. p. 213–219. *In* Proc. 1964 Conf. on Malnutrition in the Pre-school Child. Natl. Res. Coun.-Natl. Acad. Sci., Washington, D.C.

Benedict, C. R., R. J. Kohel, and M. A. Schubert. 1976. Transport of ^{14}C-assimilates to cottonseed: integrity of funiculus during seed filling stage. Crop Sci. 16:23–27.

Berardi, L. C., and J. P. Cherry. 1979a. Cottonseed protein derivatives and their potential food uses. Cott. Gin Oil Mill Press 89:14–16.

----, and ----. 1979b. Preparation and composition of co-precipitated protein isolates from cottonseed, soybean, and peanut flours. Cereal Chem. 56:95–100.

----, and ----. 1980. Textural properties of cottonseed proteins. J. Food Sci. 45:377–380, 387.

----, and ----. 1981. Functional properties of co-precipitated protein isolates from cottonseed, soybean, and peanut flours. Can. Inst. Food Sci. Technol. J. 14:283–288.

----, and V. L. Frampton. 1957. Note on gossypol and its relation to color fixation in cottonseed oil. J. Am. Oil Chem. Soc. 34:399–401.

----, and L. A. Goldblatt. 1980. Gossypol. p. 183–237. *In* I. Liener (ed.) Toxic constituents of plant foodstuffs. Academic Press, Inc., N.Y.

----, W. H. Martinez, and C. J. Fernandez. 1969. Cottonseed protein isolates: two-step extraction procedures. Food Technol. 23:75–82.

Bianchini, J. P., A. Ralaimanarivo, and E. M. Gaydow. 1982. Reversed-phase high-performance and liquid chromatography of fatty acid methyl esters with particular reference to cyclopropenoic and cyclopropanoic acids. J. High Resol. Chromat. Chromat. Comm. 5: 199–204.

Bickerstaffe, R., and A. R. Johnson. 1972. The effect of intravenous infusions of sterculic acid on milk fat synthesis. Br. J. Nutr. 27:561–570.

Blouin, F. A., and J. P. Cherry. 1980. Identification of color-causing pigments in biscuits containing cottonseed flour. J. Food Sci. 45:953–957, 961.

----, Z. M. Zarins, and J. P. Cherry. 1981a. Role of flavanoids in the production of color in biscuits prepared with wheat and cottonseed flours. J. Food Sci. 46:266–271.

----, ----, and ----. 1981b. Color. p. 21–39. *In* J. P. Cherry (ed.) Protein functionality in foods. ACS Symposium Series 147. Am. Chem. Soc., Washington, D.C.

----, ----, and ----. 1982. Discoloration of proteins by binding with phenolic compounds. p. 67–91. *In* J. P. Cherry (ed.) Food protein deterioration: mechanisms and functionality. ACS Symposium Series 206. Am. Chem. Soc., Washington, D.C.

Boatner, C. H., C. M. Hall, M. L. Rollins, and L. E. Castillon. 1947. Pigment of glands of cottonseed. II. Nature and properties of gland walls. Bot. Gaz. 108:484–494.

Bressani, R., J. E. Braham, and L. G. Elias. 1980. Human nutrition and gossypol. Food Nutr. Bull. 2:24–32.

----, and L. G. Elias. 1969. Effect of pH on the free and total gossypol and nutritive value of cottonseed and protein concentrate. Arch. Latinomer. Nutr. 19:367–379.

----, ----, A. Aquirre, and N. S. Scrimshaw. 1961. All-vegetable protein mixtures for human feeding. III. The development of INCAP vegetable mixture nine. J. Nutr. 74:201–208.

----, ----, J. E. Braham, and M. Erales. 1969. Long-term rat feeding studies with vegetable mixtures containing cottonseed flour produced by different methods. J. Agric. Food Chem. 17:1135–1138.

Brown, C. L., G. W. Cathey, and C. Lincoln. 1962. Growth and development of cotton as affected by toxaphene-DDT, methyl parathion, and calcium arsenate. J. Econ. Entom. 55: 298–301.

Caldwell, W. P. 1962. Relationship of pre-harvest environmental factors to seed deterioration in cotton. Ph.D. Dissertation. Mississippi State Univ., Mississippi State, Miss.

Canella, M., and G. Sodini. 1966. Extraction of gossypol and oligosaccharides from oilseed meals. J. Food Sci. 42:1218–1219.

Carter, M. E., J. P. Cherry, and P. A. Miller. 1979. Science and Education Administration's program on maintaining and improving the quality of cottonseed for processing. Oil Mill Gaz. 83:22–27.

Cathey, G. W. 1979. Harvest-aid chemicals and practices for cotton. Outlook Agric. 10:191–197.

Cegla, G. F., M. V. Taranto, K. R. Bell, and K. C. Rhee. 1978. Microscopic structure of textured cottonseed flour blends. J. Food Sci. 43:775–779.

Cherry, J. P. 1983. Cottonseed oil. J. Am. Oil Chem. Soc. 60:360–367.

----, and L. C. Berardi. 1982. Heat-stir denaturation of cottonseed proteins: texturization and gelation. p. 163–200. *In* J. P. Cherry (ed.) Food protein deterioration: mechanisms and functionality. ACS Symposium Series 206. Am. Chem. Soc., Washington, D.C.

----, and ----. 1983. Cottonseed. p. 187–256. *In* I. A. Wolff (ed.) CRC handbook of processing and utilization in agriculture, Vol. II: Part 2, Plant products. CRC Press, Inc., Boca Raton, Fla.

----, and M. S. Gray. 1981. Methylene chloride extraction of gossypol from cottonseed products. J. Food Sci. 46:1726–1733.

----, ----, and L. A. Jones. 1981a. A review of lecithin chemistry and glandless cottonseed as a potential commerical source. J. Am. Oil Chem. Soc. 58:903–913.

----, R. J. Kohel, L. A. Jones, and W. H. Powell. 1981b. Cottonseed quality: factors affecting feed and food uses. p. 266–283. *In* J. M. Brown (ed.) Proc. Beltwide Cott. Prod. Res. Conf., New Orleans, La. Natl. Cotton Council, Memphis, Tenn.

----, F. R. H. Katterman, and J. E. Endrizzi. 1970. Comparative studies of seed proteins of species of *Gossypium* by gel electrophoresis. Evolution 24:431–447.

----, and K. H. McWatters. 1981. Whippability and aeration. p. 149–176. *In* J. P. Cherry (ed.) Protein functionality in foods. ACS Symposium Series, No. 147. Am. Chem. Soc., Washington, D.C.

----, ----, and L. R. Beuchat. 1979a. Oilseed protein properties related to functionality in emulsions and foams. p. 1–26. *In* A. Pour-El (ed.) Functionality and protein structure. ACS Symposium Series No. 92. Am. Chem. Soc., Washington, D.C.

----, J. G. Simmons, A. H. Hyer, R. H. Garber, L. M. Carter, and H. B. Cooper. 1979b. Quality of module-stored cottonseed in California. p. 36–40. Proc. Beltwide Cott. Prod. Res. Conf., Phoenix Ariz. Natl. Cotton Council, Memphis, Tenn.

----, ----, R. J. Kohel, H. B. Cooper, M. Lehman, J. Dobbs, K. E. Fry, D. L. Kittock, and T. J. Henneberry. 1979c. Relationship of cottonseed quality to genetic and agronomic practices. Cott. Gin Oil Mill Press 80:18–21.

----, ----, and R. J. Kohel. 1978a. Potential for improving cottonseed quality by genetic and agronomic practice. p. 343–364. *In* M. Friedman (ed.) Nutritional improvement of food and feed proteins. Plenum Press, N.Y.

----, ----, and ----. 1978b. Cottonseed composition of national variety test cultivars grown at different Texas locations. p. 47–50. *In* J. M. Brown (ed.) Proc. Beltwide Cott. Prod. Res. Conf., Dallas, Texas. Natl. Cotton Council, Memphis, Tenn.

----, ----, Z. M. Zarins, J. I. Wadsworth, and C. H. Vinnett. 1978c. Cottonseed protein derivatives as nutritional and functional supplements in food formulations. p. 767–796. *In* M. Friedman (ed.) Nutritional improvement of food and feed proteins. Plenum Press, N.Y.

Choi, Y. R., E. W. Lusas, and K. C. Rhee. 1981. Succinylation of cottonseed flour: effect on the functional properties of protein isolates prepared from modified flour. J. Food Sci. 46:954–955.

----, ----, and ----. 1982a. Effects of acylation of defatted cottonseed flour with various acid anhydrides on protein extractability and functional properties of resulting protein isolates. J. Food Sci. 47:1713–1716.

----, ----, and ----. 1982b. Formulation of nondairy coffee whiteners with cottonseed protein isolates. J. Am. Oil Chem. Soc. 59:564–567.

Christiansen, M. N., and R. P. Moore. 1959. Seed coat structural differences that influence water uptake and seed quality in hard seed cotton. Agron. J. 51:582–584.

----, and ----. 1961. Temperature influence on the in vivo hydrolysis of cottonseed oil. Crop Sci. 1:385–386.

Ciegler, A., H. R. Burnmeister, R. F. Vesonder, and C. W. Hesseltine. 1981. Mycotoxins: occurrence in the environment. p. 1–50. *In* R. C. Shank (ed.) Mycotoxins and n-nitroso compounds: Environmental risks. CRC Press, Inc., Boca Raton, Fla.

Clawson, A. J., J. H. Maner, G. Gomez, Z. Flores, and J. Buitrago. 1975a. Unextracted cottonseed in diets for monogastric animals. II. The effect of boiling and oven vs. sun drying following pretreatment with a ferrous sulfate solution. J. Anim. Sci. 40:648–654.

----, ----, ----, O. Mejia, Z. Flores, and J. Buitrago. 1975b. Unextracted cottonseed in diets for monogastric animals. I. The effect of ferrous sulfate and calcium hydroxide in reducing gossypol toxicity. J. Anim. Sci. 40:640–647.

Colwick, R. F., T. H. Garner, G. D. Christenbury, G. B. Welch, R. L. Clark, J. C. Delouche, C. C. Baskin, J. W. Sorenson, L. H. Wilkes, N. K. Person, and H. W. Schroeder. 1972. Factors affecting cottonseed damage in harvesting and handling. ARS, USDA, Prod. Res. Rep. No. 135.

Conner, J. W., D. R. Krieg, and J. R. Gipson. 1972. Accumulation of simple sugars in cotton bolls as influenced by night temperatures. Crop Sci. 12:752–754.

Curley, R. G., R. A. Kepner, M. Hoover, U. U. McCutcheon, L. K. Stronberg, and E. A. Yeary. 1973. Seed cotton storage, an aid to both growers and ginners. Calif. Agric. 27: 7–9.

Damaty, S., and B. J. F. Hudson. 1975. Preparation of low-gossypol cottonseed flour. J. Sci. Fd. Agric. 26:109–115.

Davis, R. G., L. S. Bird, A. Y. Chambers, R. H. Garber, C. R. Howell, E. B. Minton, R. Sterne, and L. F. Johnson. 1981. Control of seedling diseases. p. 17–20. *In* G. M. Watkins (ed.) Compendium of cotton diseases. Am. Phytopathol. Soc., St. Paul, Minn.

Delouche, J. C. 1981. Harvest and post-harvest factors affecting the quality of cotton planting seed and seed quality evaluation. p. 289–305. *In* J. M. Brown (ed.) Proc. Beltwide Cott. Prod. Res. Conf., New Orleans, La. Natl. Cotton Council, Memphis, Tenn.

Deutshoman, A. J., and L. S. Klaus. 1960. Spectrophotometric determination of sterculic acid. Anal. Chem. 32:1809–1810.

Dewez, J. 1964. Water uptake and heat evolution by germinating cotton seed. Plant Physiol. 39:240–244.

Dieckert, J. W. and M. C. Dieckert. 1972. The deposition of vacuolar proteins in oilseeds. p. 52–85. *In* G. E. Inglett (ed.) Seed proteins. AVI Publishing Co., Inc., Westport, Conn.

----, and ----. 1976. The chemistry and cell biology of the vacuolar proteins of seeds. J. Food Sci. 41:475–482.

----, and ----. 1978. The comparative anatomy of the principal reserve proteins of seeds. *In* Abl. Akad. Wiss. DDR., Abt. Math., Naturwiss., Tech. p. 73–86.

----, R. W. Wallace, and M. C. Dieckert. 1981. Chemistry and biology of the cottonseed globulins. p. 351–355. *In* J. M. Brown (ed.) Proc. Beltwide Cott. Prod. Res. Conf., New Orleans, La. Natl. Cotton Council, Memphis, Tenn.

Dunning, J. W. 1948. Cotton hulls. p. 873–893. *In* A. E. Bailey (ed.) Cottonseed and cottonseed products: their chemistry and chemical technology. Interscience, N.Y.

Eickhoff, W. D., and M. H. Willcutt. 1978. Guidelines—seed cotton modules. p. 58–63. *In* Proc. West Cotton Prod. Res. Conf., Phoenix, Ariz.

Elmore, C. D., and H. R. Leffler. 1976. Development of cotton fruit. III. Amino acid accumulation in protein and nonprotein nitrogen fractions of cottonseed. Crop Sci. 16: 867–871.

El-Nockrashy, A. S., H. M. Mostafa, M. M. El-Fouly, and V. El-Shattory. 1976. Biochemical changes in cottonseed during development and maturity. Nahrung 20:125–132.

El-Sayed, K., A. E. Salem, and A. A. Abdel-Bary. 1978a. Utilization of cotton flour in human food. I. Effect of cotton flour on physical properties of wheat flour dough. Alex. J. Agric. Res. 26:327–332.

----, ----, and ----. 1978b. Utilization of cotton flour in human food. II. Effect of cotton protein fractions on the physical properties of wheat flour dough. Alex. J. Agric. Res. 26:609–617.

El-Zik, K. M., and L. S. Bird. 1969. Inheritance of final seedling stand ability and related components in cotton. p. 119–120. *In* J. M. Brown (ed.) Proc. Beltwide Cott. Prod. Res. Conf., New Orleans, La. Natl. Cotton Council, Memphis, Tenn.

Engleman, E. M. 1966. Ontogeny of aleurone grains in cotton embryo. Am. J. Bot. 53:231–237.

Experience Incorporated. 1978a. Feasibility study to determine specific market opportunities for cottonseed flour, fractions, and derivatives in domestic markets. Contract No. 12-14-1001-1235, Phase I. Experience, Inc., Minneapolis, Minn.

----. 1978b. The economic feasibility of modifying and operating the Lubbock cottonseed flour plant to supply potential domestic markets. Contract No. 12-14-1004-1285, Phase II. Experience, Inc., Minneapolis, Minn.

Ferguson, D., and J. H. Turner. 1971. Influence of unfilled cotton seed upon emergence and vigor. Crop Sci. 11:713–715.

Feuge, R. O., Z. M. Zarins, J. L. White, and R. L. Holmes. 1969. Titration of cyclopropene esters with hydrogen bromide. J. Am. Oil Chem. Soc. 46:185–188.

Fisher, G. S., and J. P. Cherry. 1983. Variation of cyclopropenoid fatty acids in cottonseed lipids. Lipids (in press).

----, and W. H. Schuller. 1981. Gas chromatographic analysis of cyclopropenoid acids in cottonseed oils. J. Am. Oil Chem. Soc. 58:943–946.

Fontaine, T. D., S. B. Detwiler, and G. W. Irving, Jr. 1945. Imrpovement in the color of peanut and cottonseed proteins. Ind. Eng. Chem. 37:1232–1236.

----, G. W. Irving, Jr., and K. S. Markley. 1946. Peptization of peanut and cottonseed proteins. Effects of dialysis and various acids. Ind. Eng. Chem. 38:658–662.

Freeman, D. W., R. S. Kadan, G. M. Ziegler, and J. J. Spadaro. 1979. Processing factors affecting air classification of defatted cottonseed flour for production of edible protein products. Cereal Chem. 56:452–454.

Gardner, H. K., R. J. Hron, and H. L. E. Vix. 1976. Removal of pigment glands (gossypol) from cottonseed. Cereal Chem. 53:549–560.

Gillham, F. E. M. 1969. Cotton in a hungry world. S. Afr. J. Sci. 65:173–179.

Gipson, J. R., and H. E. Joham. 1969. Influence of night temperature on growth and development of cotton (*Gossypium hirsutum* L.) IV. Seed quality. Agron. J. 61:365–367.

----, and L. L. Ray. 1970. Temperature-variety interrelationships in cotton. 1. Boll and fiber development. 2. Seed development and composition. Cott. Grow. Rev. 47:247–271.

Glade, E. H., and J. L. Ghetti. Feb. 1979. Collecting gin waste for sale. p. 24–44. *In* Cotton and wool situation. USDA-ESCS Rep. CWS-18. Washington, D.C.

Goldblatt, L. A. 1969. Aflatoxin: scientific background, control, and implications. Academic Press, N.Y.

Graham, G. G., E. Morales, G. Acevedo, J. M. Baertl, and A. Cordano. 1969. Dietary protein quality in infants and children. II. Metabolic studies with cottonseed flour. Am. J. Clin. Nutr. 22:577–587.

----, ----, ----, ----, and ----. 1970. Dietary protein quality in infants and children. III. Prolonged feeding of cottonseed flour. Am. J. Chem. Nutr. 23:165–169.

Grindley, D. N. 1950. Changes in composition of cottonseed during development and ripening. J. Sci. Food Agric. 1:147–151.

Guinn, G. 1965. Root development as affected by seed quality and environment. p. 9–11. Proc. Beltwide Cott. Prod. Mech. Conf., Atlanta, Ga.

Halloin, J. M. 1981. Weathering: changes in planting seed quality between ripening and harvest. p. 286–289. *In* J. M. Brown (ed.) Proc. Beltwide Cotton Prod. Res. Conf., New Orleans, La. Natl. Cotton Council, Memphis, Tenn.

----. 1982. Localization and changes in catechin and tannins during development and ripening of cottonseed. New Phytol. 90:651–657.

----, and A. A. Bell. 1979. Production of nonglandular terpenoid aldehydes within diseased seeds and cotyledons of *Gossypium hirsutum* L. J. Agric. Food Chem. 27:1407–1409.

Harden, M. L., and S. P. Yang. 1975. Protein quality and supplementary value of cottonseed flour. J. Food Sci. 40:75–77.

Harrison, J. R. 1977. Review of extraction process: emphasis cottonseed. Oil Mill Gaz. 82: 16–24.

Hess, D. C. 1976. Prospects for glandular cottonseed. Oil Mill Gaz. 82:22–26.

----. 1977a. Genetic improvement of gossypol-free cotton varieties. Cereal Foods World 22: 98–105.

----. 1977b. Selecting for increased seed density in cotton. p. 84–86. *In* J. M. Brown (ed.) Proc. Beltwide Cott. Prod. Res. Conf., Atlanta, Ga. Natl. Cotton Council, Memphis, Tenn.

Hoffman, W. H., and J. C. Arthur. 1952. Cottonseed meal as a tire cord-to-rubber adhesive. Rubber Agric. 71:354–356.

Hogan, J. T., and J. C. Arthur. 1951a. Preparation and utilization of cottonseed meal glue for plywood. J. Am. Oil Chem. Soc. 28:20–23.

----, and ----. 1951b. Cottonseed and peanut meal glues: permanence of plywood glue joints as determined by interior and exterior accelerated cyclic service tests. J. Am. Oil Chem. Soc. 28:272–274.

----, and ----. 1952. Cottonseed and peanut meal glues. Resistance of plywood bonds to chemical reagents. J. Am. Oil Chem. Soc. 29:16–18.

Hunter, R. E., and J. T. Presley. 1963. Morphology and histology of pinched root tips of *Gossypium hirsutum* L. seedlings grown from deteriorated seeds. Can. J. Plant Sci. 43: 146–150.

Ibragimov, A. P., P. Tursunbaev, A. V. Tuichiev, and Sh. Yunuskhanov. 1974. Study of 7S globulin subunits from cotton seeds. Biokhimiya 39:225–229.

Johnson, A. R., J. A. Pearson, F. S. Shenstone, and A. C. Fogrty. 1967. Inhibition of stearic to oleic acid by cyclopropene fatty acids. Nature 214:1244–1245.

Jones, D. B., and F. A. Csonka. 1925. Proteins of the cottonseed. J. Biol. Chem. 64:673–683.

Jones, L. A. 1981. Natural antinutrients of cottonseed protein products. p. 77–98. *In* R. L. Ory (ed.) Antinutrients and natural toxicants in foods. Food and Nutrition Press, Inc., Westport, Conn.

Kadan, R. S., D. W. Freeman, G. M. Ziegler, and J. J. Spadaro. 1979. Air classification of defatted, glanded cottonseed flours to edible protein product. J. Food Sci. 44:1522–1524.

----, ----, ----, and ----. 1980. Protein displacement during classification of glanded cottonseed. J. Food Sci. 45:1566–1569, 1572.

Kajimoto, G., H. Yoshida, A. Shibahara, and S. Yamashoji. 1979. Changes in the composition of lipids and fatty acids in cottonseeds during maturation. Nippon Nageikagaku Kaishi 53:317–320.

Karavaeva, N. N. 1972. Study of structure of cottonseed globulin A. Biochemistry [Translation] Biokhimiya 37:603–608.

Kepner, R. A., and R. G. Curley. 1976. Handling seed cotton modules without pellets. Costs of hauling from field to storage. Calif. Agric. 30:6–8.

King, E. E., and G. E. Lamkin. 1979. Uniform quality cottonseed for laboratory and field use. p. 32. *In* J. M. Brown (ed.) Proc. Beltwide Cott. Prod. Res. Conf., Phoenix, Ariz. Natl. Cotton Council, Memphis, Tenn.

Kircher, H. W. 1964. The addition of mercaptans to methyl sterculate and sterculene: a hypothesis concerning the nature of the biological activity exhibited by cyclopropene derivatives. Am. Oil Chem. Soc. 41:4–8.

Kohel, R. J., and J. P. Cherry. 1983. Variation of cottonseed quality with stratified harvests. Crop Sci. 23:1119–1124.

Krieg, D. R., and S. N. Bartee. 1975. Cottonseed density: associated germination and seedling emergence properties. Agron. J. 67:343–347.

----, and J. D. Carroll. 1978. Cotton seedling metabolism as influenced by germination temperature, cultivar, and seed physical properties. Agron. J. 70:21–25.

----, J. R. Gipson, and L. W. Barnes. 1973. The chemical composition of cotton bolls and seeds developed under controlled night temperatures. p. 42–45. *In* J. M. Brown (ed.) Proc. Beltwide Cott. Prod. Res. Conf., Phoenix, Ariz. Natl. Cotton Council, Memphis, Tenn.

Kromer, G. W. 1977. Current status and future market potential for cottonseed. p. 1–9. *In* Glandless cotton: its significance, status, and prospects. Proc. Conf. ARS, USDA, Dallas, Texas.

Kuchenkova, M. A., E. F. Redina, N. L. Ovchimnikova, and P. Kh. Yuldashev. 1977. The globulins of cottonseeds. XII: The structure of the 7S globulin. Chem. Nat. Compd. (USSR) 13:570–572.

----, ----, and P. Kh. Yuldashev. 1981. A study of the globulin of cottonseeds. Isolation and characterization of a glycopeptide of the 7S globulin. Chem. Nat. Compd. (USSR) 17: 171–173.

Lawhon, J. T., and C. M. Cater. 1971. Effect of processing method on the yields and functional properties of protein isolates from glandless cottonseed. J. Food Sci. 36:372–377.

----, ----, and K. F. Mattil. 1976. Evaluation of the food use potential of sixteen varieties of cottonseed. J. Am. Oil Chem. Soc. 54:75–80.

----, D. W. Hensley, D. Mulsow, and K. F. Mattil. 1978. Optimization of protein isolate production from soy flour using industrial membrane systems. J. Food Sci. 43:361–364, 369.

----, S. H. C. Lin, D. W. Hensley, C. M. Cater, and K. F. Mattil. 1977b. Processing whey-type byproduct liquids from cottonseed protein isolation with ultrafiltration and reverse osmosis membranes. J. Food Process. Eng. 1:15–35.

----, L. J. Manak, and E. W. Lusas. 1980. An improved process for isolation of glandless cottonseed protein using industrial membrane systems. J. Food Sci. 45:197–199, 203.

----, D. Mulsow, C. M. Cater, and K. F. Mattil. 1977a. Production of protein isolates and concentrates from oilseed flour extracts using industrial ultrafiltration and reverse osmosis. J. Food Sci. 42:389–394.

Lee, L. S., and L. A. Goldblatt. 1981. Contributions of Walter A. Pons, Jr., to development of methodology for mycotoxins. 1981. J. Am. Oil Chem. Soc. 58:928A–930A.

----, and T. E. Russell. 1981. Distribution of aflatoxin-containing cottonseed within intact locks. J. Am. Oil Chem. Soc. 58:27–29.

Leffler, H. R. 1976. Development of cotton fruit. I. Accumulation and distribution of dry matter. Agron. J. 68:855–857.

----. 1980. Leaf growth and senescence. p. 133–142. *In* J. D. Hesketh and J. W. Jones (ed.) Predicting photosynthesis for economic models. Vol. II. CRC Press, Inc., Boca Raton, Fla.

----, and R. D. Williams. 1983. Seed density classification influences germination and seedling growth of cotton. Crop Sci. 23:161–165.

----, C. D. Elmore, and J. D. Hesketh. 1977. Seasonal and fertility-related changes in cottonseed protein quantity and quality. Crop Sci. 17:953–956.

----, W. R. Meredith, Jr., and J. M. Chandler. 1978. Canopy physiology and seasonal patterns affect the composition and yield capacity of cottonseed. Agron. Abst. 70:79–80.

Lusas, E. W., J. T. Lawhon, S. P. Clark, S. W. Matlock, W. W. Meinke, D. W. Mulsow, K. C. Rhee, and P. J. Wan. 1977. Potential for edible protein products from glandless cottonseed. p. 31–43. *In* Glandless cotton: its significance, status, and prospects. Proc. Conf., ARS, USDA, Dallas, Texas.

Maleki, P. 1966. Microenvironmental influence on cottonseed deterioration in the field. M.S. Thesis, Mississippi State Univ., Mississippi State, Miss.

Manak, L. J., J. T. Lawhon, and E. W. Lusas. 1980. Functioning potential on soy, cottonseed, and peanut protein isolates produced by industrial membrane systems. J. Food Sci. 45:236–238, 245.

Markman, A. L., and V. P. Rzhekhin. 1965. Gossypol and its derivatives. IPST Press Binding: Wiener Bindery Ltd., Jerusalem, Israel.

Marshall, H. F., M. A. Shirer, and J. P. Cherry. 1984. Characterization of glandless cottonseed storage proteins by SDS-polyacrylamide gel electrophoresis. Cereal Chem. (in press).

Martinez, W. H. 1979. Functionality of vegetable proteins other than soy. J. Am. Oil Chem. Soc. 56:280–284.

----, L. C. Berardi, and L. A. Goldblatt. 1970. Potential of cottonseed: products, composition, and use. p. 248–261. Third Internat. Congr. Food Sci. and Technol. SOS/70 Proceed. Instit. of Food Technol., Chicago, Ill.

----, and D. T. Hopkins. 1975. Cottonseed protein products: variation in protein quality with product and process. p. 355–374. *In* M. Friedman (ed.) Nutrition quality of foods and feed. Part II. Quality factors plant breeding, composition, processing, and anti-nutrients. Marcel Dekker, N.Y.

Mathews, J. L. 1981. World and U.S. oilseeds and products outlook. p. 183. *In* Agric. Outlook, Commit. on Agric., Nutr., and Forestry. U.S. Government Printing Office, Washington, D.C.

Mayne, F. C., J. A. Harris, R. A. Pittman, and E. L. Skau. 1966. Methods for the determination of cyclopropenoid fatty acids. VII. The dilution HBr-titration technique as a general method. J. Am. Oil Chem. Soc. 3:319–524.

McMeans, J. L., V. T. Walhood, and L. M. Carter. 1966. Effects of greenpick, defoliation, and desiccation practices on quality and yield of cotton, *Gossypium hirsutum.* Agron. J. 58:91–94.

McMichael, S. C. 1959. Hopi cotton, a source of cottonseed free of gossypol pigments. Agron. J. 51:630.

----. 1960. Combined effects of glandless genes gl_2 and gl_3 on pigment glands in the cotton plant. Agron. J. 52:385–386.

McWatters, K. H., and J. P. Cherry. 1981. Emulsification: vegetable proteins. p. 217–242. *In* J. P. Cherry (ed.) Protein functionality in foods. ACS Symposium Series No. 147. Am. Chem. Soc., Washington, D.C.

Meinke, W. W. 1952. Food uses for cottonseed. Texas A&M College System Res. Rep. No. 34. Texas A&M Univ., College Station.

Meredith, W. R., Jr., and R. R. Bridge. 1973. Yield, yield component and fiber property variation of cotton (*Gossypium hirsutum* L.) within and among environments. Crop Sci. 13:307–312.

Milner, M. 1965. Problems and opportunities in utilizing cottonseed proteins to meet world protein deficits. p. 8–13. *In* Proc. 7th Conf. Cottonseed Protein Conc. USDA ARS Rep. 72-38.

----. 1969. Status of development and use of some unconventional proteins. p. 97–104. *In* M. Milner (ed.) Protein-enriched cereal foods for world needs. Assoc. Cereal Chem., St. Paul, Minn.

----. 1980. Guideline for edible cottonseed protein flours and related products. Food Nutr. Bull. 2:51–54.

Minton, E. B., and J. R. Supak. 1980. Effects of seed density on stand, *Verticillium* wilt, and seed and fiber characteristics. Crop Sci. 20:345–347.

Morgan, P. W. 1979. Plant growth regulators. Texas Agric. Progr. 25:22–23.

Newberne, P. M., and A. E. Rogers. 1981. Animal toxicity of major environmental mycotoxins. p. 51–106. *In* R. C. Shank (ed.) Mycotoxins and n-nitroso compounds: environmental risks. CRC Press, Inc., Boca Raton, Fla.

Olsen, R. L. 1973. Evaluation of LCP cottonseed and flour. Oil Mill Gaz. 77:7–8.

Osborne, J. B., and C. W. Voorhees. 1894. The proteins of cottonseed. J. Am. Chem. Soc. 14:778–785.

Ovchinnikova, N. L., M. A. Kuchenkova, T. D. Kasymova, and P. Kh. Yuldashev. 1977. The globulins of cottonseed. XI. The structure of the chymotryptic peptides of the 7S globulin. Chem. Nat. Compd (USSR) 13:566–569.

Pandey, S. N., and N. Thejappa. 1975. Study on the relationship between oil, protein, and gossypol in cottonseed kernels. J. Am. Oil Chem. Soc. 52:312–315.

Park, M.-S., and G. U. Liepa. 1982. Effects of dietary protein and amino acids on the metabolism of cholesterol-carrying lipoproteins in fats. J. Nutr. 112:1892–1898.

Paxton, K. W., and D. L. Roberts. 1973. Economic feasibility of storing seed cotton in modules under Louisiana conditions. Field storage processing costs. Dep. Agric. Econ. Agribus., Los Angeles State Univ., Agric. Mech. Coll. Agric. Exp. Stn. Rep. No. 456.

Peacock, H. A., and B. S. Hawkins. 1970. Effect of seed source on seedling vigor, yield, and lint characteristics of upland cotton, *Gossypium hirsutum* L. Crop Sci. 10:667–670.

Phelps, R. A., J. P. Cherry, A. H. Hyer, G. C. Cavanaugh, G. A. Harper, J. E. Jernigan, D. C. Hess, and D. G. Lorance. 1979. Advancements in cottonseed quality—industry's viewpoint. Cott. Gin Oil Mill Press 80:14–15.

----, F. S. Shenstone, A. R. Kemmerer, and R. J. Evans. 1965. A review of cyclopropenoid compounds: biological effects of some derivatives. Poult. Sci. 44:358–394.

Podgornov, G. M., M. A. Kuchenkova, and P. Kh. Yuldashev. 1974. Cottonseed globulins. 2. N-terminal amino acids. Chem. Nat. Compd. (USSR) 10:855.

Pons, W. A. 1977. Gossypol analysis; past and present. J. Assoc. Off. Anal. Chem. 60:252–259.

----, L. C. Berardi, and V. L. Frampton. 1959. Kinetic study of gossypol fixation in cottonseed oil. J. Am. Oil Chem. Soc. 36:337–339.

----, and P. H. Eaves. 1971. Aqueous acetone extraction of cottonseed. U.S. Patent 3,557,168.

----, and A. O. Franz. 1978. High pressure liquid chromatographic determination of aflatoxins in peanut products. J. Assoc. Off. Anal. Chem. 61:793–800.

Pope, O. A., and J. O. Ware. 1945. Effect of variety, location, and season on oil, protein, and fuzz of cottonseed and on fiber problems of lint. USDA Tech. Bull. 903.

Ramey, H. H. 1974. A systems analysis approach to establishing research objectives for control of aflatoxin production in cottonseed. Phytopathology 64:1451–1454.

Reber, E. F. 1981. Safety of low gossypol cottonseed kernels. J. Food Sci. 46:593–596.

----, and R. E. Pyke. 1980. Sub-acute toxicity studies of glandless cottonseed kernels fed to rats. J. Food Safety 2:87–95.

Redina, E. F., M. A. Kuchenkova, L. G. Petrosyan, and P. Kh. Yuldashev. 1977. The globulins of cottonseeds. X. Structures of tryptic peptides of the 7S globulin. Chem. Nat. Compd. (USSR) 13:563–565.

Rhee, K. C., C. K. Kuo, and E. W. Lusas. 1981. Texturization. p. 51–88. *In* J. P. Cherry (ed.) Protein functionality in foods. ACS Symposium Series No. 147. Am. Chem. Soc., Washington, D.C.

Roark, B., T. R. Pfrimmer, and M. E. Merkl. 1963. Effects of some formulations of methyl parathion, toxaphene, and DDT on the cotton plant. Crop Sci. 3:338–341.

Roberts, D. L., K. W. Paston, and R. L. Gill. 1973. The module system: a new approach in storing seed cotton. Louisiana Agric. 16:4–5.

Roehm, J. N., D. J. Lee, J. H. Wales, S. D. Polityka, and P. D. Sinnhuber. 1970. The effect of the dietary sterculic acid on the hepatic lipids of rainbow trout. Lipids 5:80–84.

Roncadori, R. W., O. L. Brooks, and C. E. Perry. 1972. Effect of field exposure on fungal invasion and deterioration of cotton seed. Phytopathology 62:1137–1139.

----, S. M. McCarter, and J. L. Crawford. 1971. Influence of fungi on cotton seed deterioration prior to harvest. Phytopathology 61:1326–1328.

Rosenblum, D. 1980. Glandless cottonseed opens new route for developing protein-fortified foods. Food Prod. Develop. 14:34, 38, 40, 72.

Rossi-Fanelli, A., E. Antonini, M. Brunori, M. R. Bruzzesi, A. Caputo, and F. Satriana. 1964. Isolation of a monodisperse protein fraction from cottonseeds. Biochem. Biophys. Res. Commun. 15:110–115.

Russell, M. B. 1957. Physical properties. p. 31–38. *In* A. Stefferud (ed.) Soils. USDA Yearbk Agric., Washington, D.C.

Russell, T. E., T. F. Watson, and G. F. Ryan. 1976. Field accumulation of aflatoxin in cottonseed as influenced by irrigation termination dates and pink bollworm infestation. Appl. Environ. Microbiol. 31:711–713.

St. Angelo, A. J., and A. M. Altschul. 1964. Lipolysis and the free fatty acid pool in seedlings. Plant Physiol. 39:880–883.

Scrimshaw, N. S. 1980. A look at the Incaparina experiment in Guatemala. The background and history of Incaparina. Food Nutr. Bull. 2:1–2.

Shadrina, T. Y., T. S. Yunusov, and P. Kh Yuldashev. 1979. A study of the globulins of cottonseed. XX. Stability of the quaternary structure of the 11S globulin. Khim. Prir. Soedin. 4:554–557.

Sheehan, E. T., D. L. Schneider, and M. G. Vavich. 1972. Improved halphen method for measuring cyclopropenoid fatty acids. J. Agric. Food Chem. 22:119–121.

Simmons, R. G., J. R. Green, C. A. Payne, P. J. Wan, and E. W. Lusas. 1980. Cottonseed and soy protein ingredients in self-serve frozen desserts. J. Food Sci. 45:1505–1508.

Simpson, D. M., and B. M. Stone. 1935. Viability of cottonseed as affected by field conditions. J. Agric. Res. 50:435–477.

Smith, F. H. 1972. Effect of gossypol bound to cottonseed protein on growth of weanling rats. J. Agric. Food Chem. 20:803–804.

Smith, K. J. 1970. Practical significance of gossypol in food formulation. J. Am. Oil Chem. Soc. 47:448–450.

Sood, D. R., V. Kumar, and K. S. Dhindsa. 1976. Composition of cottonseed as affected by N, P, and K application. Agrochimica 20:77–81.

Spadaro, J. J., and H. K. Gardner. 1979. Food uses for cottonseed protein. J. Am. Oil Chem. Soc. 56:422–424.

Srikantia, S. G., and S. Sahgal. 1968. Use of cottonseed protein in protein-calorie malnutrition. Am. J. Clin. Nutr. 21:212–216.

Stansbury, M. F., C. L. Hoffpauir, and T. H. Hopper. 1953a. Influence of variety and environment on the iodine value of cottonseed oil. J. Am. Oil Chem. Soc. 30:120–123.

----, A. F. Cucullu, and G. T. Den Hartog. 1954. Cottonseed contents variation: influence of variety and environment on oil content of cottonseed kernels. Agric. Food Chem. 2: 692–696.

Stansbury, M. F., W. A. Pons, and G. T. Den Hartog. 1956. Relations between oil, nitrogen, and gossypol in cottonseed kernels. J. Am. Oil Chem. Soc. 33:282–286.

----, ----, and C. L. Hoffpauir. 1953b. Phosphorus compounds in cottonseed kernels. Influence of variety of cottonseed and environment. Agric. Food Chem. 1:75–78.

Stewart, J. Mc D. 1980. Integrated developmental events and their response to environment in cotton bolls. p. 322–341. *In* J. M. Brown (ed.) Proc. Beltwide Cott. Prod. Res. Conf., St. Louis, Mo. Natl. Cotton Council, Memphis, Tenn.

Taranto, M. V., G. F. Cegla, and K. C. Rhee. 1978a. Morphological, ultrastructural and rheological evlauation of soy and cottonseed flours texturized by extrusion and nonextrusion processing. J. Food Sci. 43:973–979, 984.

----, ----, K. R. Bell, and K. C. Rhee. 1978b. Textured cottonseed and soy flours—microscopic analysis. J. Food Sci. 43:767–771.

----, W. W. Meinke, C. M. Cater, and K. F. Mattil. 1975. Parameters affecting production and character of extrusion texturized defatted glandless cottonseed meal. J. Food Sci. 40: 1264–1269.

Terrell, R. N., J. A. Brown, Z. L. Carpenter, K. F. Mattil, and C. W. Monagle. 1979. Effects of oilseed proteins, at two replacement levels on chemical, sensory, and physical properties of frankfurters. J. Food Sci. 44:865–868.

----, R. L. Swasdee, P. J. Wan, and E. W. Lusas. 1981. Cottonseed proteins in frankfurters: effect on pH, cured color, and sensory and physical properties. J. Food Sci. 46:845–849.

Thomas, M. R., J. Ashby, S. M. Sneed, and L. M. O'Rear. 1979. Minimum nitrogen requirement from glandless cottonseed protein for nitrogen balance in college women. J. Nutr. 109:397–409.

Thomson, J. R. 1979. An introduction to seed technology. Wiley, N.Y.

Tupper, G. R., L. E. Clark, and O. R. Kunze. 1970. The measurement of certain physical characteristics related to rapid germination and seedling vigor in cotton seed. Proc. Assoc. Off. Seed Anal. 60:138–148.

----, O. R. Kunze, and L. H. Wilkes. 1971. Physical characteristics of cottonseed related to seedling vigor and design parameters for seed selection. Trans. Am. Soc. Agric. Eng. 14: 890–893.

Turner, J. H., and D. Ferguson. 1972. Field performance of cotton grown from filled and partially filled seeds. Crop Sci. 12:868–871.

----, H. H. Ramey, and S. Worley. 1976a. Influence of environment on seed quality of four cotton cultivars. Crop Sci. 16:407–409.

----, ----, and ----. 1976b. Relationship of yield, seed quality, and fiber properties in upland cotton. Crop Sci. 16:578–580.

Van Waalwijkn van Doorn, J. J. L. 1982. U.S. sun oil exports expected to raise. J. Am. Oil Chem. Soc. 59:588A–597A.

Van Wyck, P. 1948. Cotton linters. p. 894–905. *In* A. E. Bailey (ed.) Cottonseed and cottonseed products: their chemistry and chemical technology. Interscience, N.Y.

Vix, H. L. E., H. P. Dupuy, and M. G. Lambou. 1969. Critical evaluation of the use of acetone in solvent extraction processes. p. 62–69. *In* Conference on protein-rich food products from oil seeds. New Orleans, La. USDA ARS Rep. 72-71.

----, P. H. Eaves, H. K. Gardner, and M. G. Lambou. 1971. Degossypolized cottonseed flour —the liquid cyclone process. J. Am. Oil Chem. Soc. 48:611–615.

----, J. J. Spadaro, C. H. Murphy, R. M. Persell, E. F. Pollard, and E. A. Gastrock. 1949. Pilot-plant fractionation of cottonseed. II. Differential settling. J. Am. Oil Chem. Soc. 26:526–530.

----, ----, R. D. Westbrook, A. J. Crovetto, E. F. Pollard, and E. A. Gastrock. 1947. Prepilot plant mixed-solvent flotation process for separating pigment glands from cottonseed meats. J. Am. Oil Chem. Soc. 24:228–236.

Walhood, V. T. 1956. A method of reducing the hard seed problem in cotton. Agron. J. 141–142.

Walhood, V. T., and R. E. Johnson. 1976. Inception of solar radiation by a constant population of cotton plants. p. 70–71. *In* J. M. Brown (ed.) Proc. Beltwide Cott. Prod. Res. Conf., Las Vegas, Nev. Natl. Cotton Council, Memphis, Tenn.

Wanjura, D. F., E. B. Hudspeth, Jr., and J. D. Bilbro, Jr. 1967. Temperature-emergence relations of cottonseed under natural diurnal fluctuation. Agron. J. 59:217–219.

Wanjura, D. F., E. G. Hudspeth, Jr., and J. D. Bilbro, Jr. 1969. Emergence time, seed quality, and planting depth effects on yield and survival of cotton (*Gossypium hirsutum* L.). Agron. J. 61:63–65.

----, and E. B. Minton. 1981. Delayed emergence and temperature influences on cotton seedling vigor. Agron. J. 73:594–597.

Watson, H., and J. D. Helmer. 1964. Cottonseed quality as affected by the ginning process—a progress report. ARS USDA Publ. 42-107.

Wiles, A. B., and J. T. Presley. 1960. Seed deterioration as a factor in nub-root production in cotton. Plant Dis. Rep. 44:472–473.

Wilkes, L. H. 1978. Seed cotton storage: effects on seed quality. p. 215–217. *In* J. M. Brown (ed.) Proc. Beltwide Cott. Prod. Res. Conf., Dallas, Texas. Natl. Cotton Council, Memphis, Tenn.

Wilkes, L. H., and T. E. Corley. 1968. Planting and cultivation. p. 117–149. *In* F. C. Elliot, M. Hoover, and W. K. Porter (ed.) Advances in production and utilization of quality cotton: principles and practices. Iowa State Univ. Press, Ames.

Wise, R. P. 1980. The case of Incaparina in Guatemala. Food Nutr. Bull. 2:3–8.

Yatsu, L. Y. 1965. The ultrastructure of cotyledonary tissue from *Gossypium hirsutum* L. seeds. J. Cell Biol. 25:193–199.

Zarins, Z. M., and J. P. Cherry. 1981. Storage proteins of glandless cottonseed flour. J. Food Sci. 46:1855–1859, 1867.

Ziprin, Y. A., K. S. Rhee, Z. L. Carpenter, R. L. Hostetler, R. N. Terrell, and K. C. Rhee. 1981. Glandless cottonseed, peanut and soy protein ingredients in ground beef patties: effect on rancidity and other quality factors. J. Food Sci. 46:58–61.